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ARE *LEUCOCYTOZOON SAKHAROFFI* SAMBON AND *LEUCOCYTOZOON BERESTNEFFI* SAMBON SYNONYMOUS?[1]

BY A. MURRAY FALLIS

Abstract

The size of the gametocytes of a leucocytozoon in young crows shows a wide variation in birds obtained in different localities. The host cell nucleus of infected cells may be pushed to one side, almost surround the parasite, or be intermediate between these extremes. Previous descriptions, given by various authors, of *Leucocytozoon sakharoffi* and *L. berestneffi* show similar differences in these characters. *L. berestneffi* is considered therefore to be a synonym of *L. sakharoffi*.

Sakharoff (9) appears to have given us the first description of a leucocytozoon in a host cell that retained its spherical shape. He described *Leucocytozoon* in the raven and the rook and distinguished between the parasites in these two birds by the appearance of the nuclei in the infected host cells. The nucleus of the infected host cell of the raven assumed the appearance of a narrow band that almost encircled the parasite, whereas in the rook the nucleus was not altered but merely pushed to one side. Both types of parasites were recorded also by Berestneff (1) in the leucocytes of a raven and a magpie.

These parasites were given specific status by Sambon (10) who named the form in the raven *L. sakharoffi* and described it briefly. He noted the tendency of the nucleus of the host cell to surround the parasite. He named the form in the magpie, *Pica pica*, *L. berestneffi*, but it was not described until 1937 by Coatney and Roudabush (4) from specimens observed in the blood of *Pica pica hudsonia*. It was reported from other species of the genus *Pica* by Leger (7), Böing (2), and Wingstrand (11).

A third species, *L. zuccarelli*, observed by Leger (6) in *Corvus corone*, was placed in synonomy with *L. sakharoffi* by Coatney and West (5).

L. berestneffi has been reported in a crow by Coatney and Jellison (3). Morgan and Waller (8) reported *L. sakharoffi* from a crow in Wisconsin although they thought it might be *L. berestneffi*.

The sizes of the gametocytes of *L. sakharoffi* and *L. berestneffi*, as recorded by different authors, show rather wide variations. Coatney and Roudabush

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(4) reported that *L. berestneffi* was smaller than *L. sakharoffi* but Wingstrand (11) found the opposite. Wingstrand stated, ". . . . that size cannot be used as a characteristic for separating the two species in fixed blood smears even though a difference may exist *in vivo*." Even in the single species *L. sakharoffi*, the measurements published by Leger (6) and by Coatney and West (5) are smaller than those given by Wingstrand (11). The latter author noted that the extremes were covered by the values given by the other authors but the means differed. Wingstrand concluded, ". . . . . the mean values cannot be too extensively used as *differentia specifica*. Different mean values can be obtained in two preparations from the same host animal."

Wingstrand thought the form of the host cell nucleus might be a more reliable difference between these two species. In his preparations the host cell nucleus more nearly surrounded *L. berestneffi* although similar nuclei were found in cells containing *L. sakharoffi*. Moreover Berestneff (1) figured parasites from *Corvus corone* that almost surrounded the host cell nuclei. Wingstrand concludes, "it seems as if this difference were a little uncertain too. In any case, the two species must be regarded as very closely related."

In a study of leucocytozoon infections in crows in Ontario, a large number of mature gametocytes have been examined in stained blood smears, taken from young crows, with new infections, from widely separated localities. A number of the parasitized cells were magnified 1400 times, projected on a screen, and the areas of the parasites and host cells were measured by means of a planimeter, and the appearance of the host cell nuclei was noted. It was apparent (Fig. 1) that some host cell nuclei almost surrounded the parasites, others were pushed to one side, and some occupied a position between these extremes. The size of the parasites, host cells, and host cell nuclei differed also within the same smear (Table I) although on the average smaller measurements were obtained in those cases in which the nuclei of the host cells were located at one side of the parasite. The presence of parasites of two extremes of size and extremes in the appearance of the host cell nuclei might suggest a mixed infection. This is most unlikely, however, as parasites intermediate in size between the extremes and host cell nuclei intermediate in shape and position were present in all of the smears. Moreover it is highly improbable that birds from widely separated localities would have the same mixed infections at approximately the same time. Furthermore a comparison of the descriptions of other authors reveals wide differences in these characters.

These variations in the size and appearance of the parasites and host cell nuclei lead to the conclusion that the species that has been found in crows might be either *L. sakharoffi* or *L. berestneffi* depending on which particular cells are considered typical. In the absence of other characters to separate these species it would appear therefore that they are similar and consequently *L. berestneffi* should be regarded as a synonym of *L. sakharoffi*.

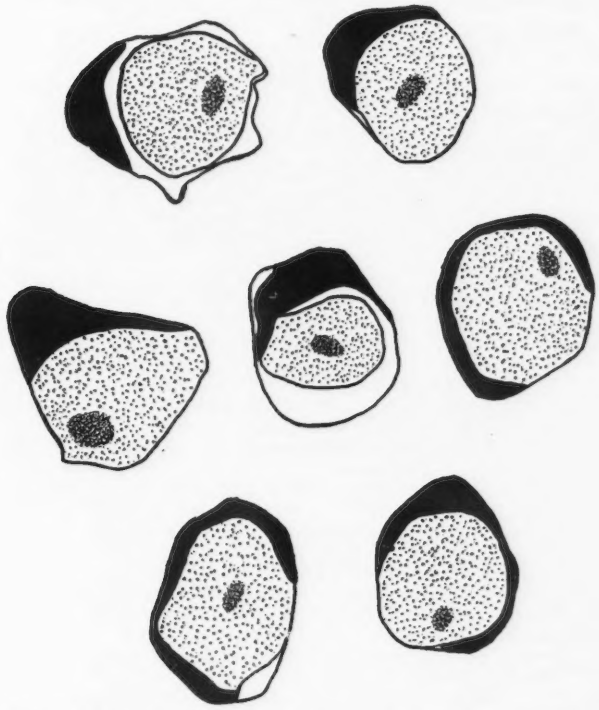

FIG. 1. *Outlines of host cells infected with L. sakharoffi to show variations in the size of the parasite and the appearance of the host cell nuclei.*

TABLE I

SIZE OF HOST CELLS, HOST CELL NUCLEI, AND PARASITES IN DRIED AND STAINED BLOOD SMEAR FROM A CROW

Nucleus surrounding parasite			Nucleus to one side of parasite		
Host cell	Host cell nucleus	Parasite	Host cell	Host cell nucleus	Parasite
Area in sq. mm. $\times 10^{-5}$			Area in sq. mm. $\times 10^{-5}$		
8.5	1.8	6.5	9.5	1.5	6.1
8.3	2.0	5.7	8.1	1.6	6.6
8.8	1.8	6.7	6.1	1.1	4.8
9.6	2.6	7.4	9.1	2.1	7.0
8.3	2.0	5.9	9.8	2.3	7.4
8.2	2.6	6.0	8.6	1.4	6.6
7.8	1.8	6.0	7.2	1.1	5.8
7.2	1.7	5.6	10.0	2.1	6.1
7.4	1.5	6.1	8.7	2.1	6.0
11.5	2.9	8.8	6.2	1.4	5.4
Av. 8.6	2.1	6.5	8.3	1.7	6.2

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MOUSE EPICYTES AND THEIR INTRAMURAL PROCESSES[1]

By Charles C. Macklin

Abstract

Thin paraffin sections of mouse lungs fixed by bronchial filling and stained intensely with iron haematoxylin show numerous and conspicuous epicytes of varying size, form, internal features, and density. Many have a dark lacelike cytoplasm with numerous small clear vacuoloids and a rounded opaque nucleus, while others stain more lightly and have large irregular vacuoloids, and a generally achromatic nucleus. Between these apparent extremes are graded intermediate forms. These epicytes may be arranged in a series perhaps connoting a functional or age sequence. Metabolic, endocrinic, and exocrinic roles have been suggested for them as well as the commonly attributed phagocytic function. The nuclei are often indented by contiguous vacuoloids. In partitions between alveoli, epicytes may have two, three, or even more air faces. Most of them are in the angles where septa between contiguous alveoli join. In many epicytes an extension may be seen arising from a larger nucleated part; and not infrequently two such extensions appear in the section. These processes may be more or less blunt and short, or long and thin; and their ends, of varying area, often form part of the wall of an alveolus, lying directly against the air space. On some of the narrow free ends were seen irregular spicules, which may be rudimentary cilia. In cross sections the processes are of circular or oval outline and are partially or completely buried in the connective tissue of the alveolar wall. Like the juxtanuclear part of the epicyte, the processes are bounded by a delicate cell membrane and show mitochondria in the cytoplasm between the vacuoloids. These processes presumably afford anchorages for the cells to the alveolar walls, and also surfaces through which nutriment may be absorbed from the contiguous capillaries. Epicytes that abut upon the pleura, interlobular septa, or sheaths of air and blood channels usually have only one air face and often appear without a process, partly sunken into the connective tissue, with a mass of frothy cytoplasm bulging into the alveolar space; but more rarely a process is visible arising from them. Release of epicytes from the alveolar walls may be possible, to create the relatively scarce free foam cells. Curious extended epicytes, some looking like dumb-bells, were often found.

Introduction

The pulmonic alveolar epicyte (Clara, 1936 (1)) has been recognized for many years under various designations, as septal cell, niche cell, little granular cell, and residual alveolar epithelial cell. Conventionally regarded as originating from the entodermal epithelium of the laryngotracheal groove (Macklin, 1946 (12); Rosin, 1947 (22)), it is thought by certain writers to be of mesodermal origin (Loosli, 1938 (9); Waddell, 1949 (24)). Although not conspicuous in ordinary sections, it may be made so by special technics, whereupon its numbers appear so impressive that it has been visualized as the cellular unit of a diffuse suborgan within the lung (Sjöstrand and Sjöstrand, 1938 (23); Hayek, 1942 (8)). It has been credited with phagocytic potency; but other functions have been suggested, and its special morphology and relations invite further study in this regard. It is viewed as having latent oncogenic power (Cowdry, 1938 (2, p. 364); Macklin, 1938 (10); Grady and Stewart, 1940 (4)).

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Contribution from the Department of Histological Research, Faculty of Medicine, the University of Western Ontario, London, Ont.

Categories

Epicytes may be divided into two groups according as they occur in marginal or partitional alveolar walls (Macklin, 1945 (11); 1946 (12)). The marginal walls are really the bases of certain alveoli and lie upon the connective tissue of the bronchi, blood vessels, pleura, and interlobular septa. Marginal epicytes have usually but one air face, the opposite end resting on connective tissue as in one-layered epithelium (Fig. 20). The partitional walls intervene between alveoli and have air on both sides. Their epicytes go right through the wall and have two or more air faces (Fig. 12). In this feature they are unique as epithelial derivatives. The partitional epicytes make up the larger group, and are those mainly discussed in this paper.

Aside from these distinctions, epicytes have a great variety of form, even when prepared by the same fixing and staining technic; but this pleomorphism is even more marked when the epicytes have been prepared by different methods. Their morphologic inequalities arising from the use of diverse fixation technics, such as bronchial filling, vascular perfusion, or immersion, have been emphasized (Macklin, 1946 (12)). Their most distinctive feature is the equipment of vacuoloids (Macklin, 1947 (13); 1949 (17); 1949 (19); 1949 (20)), which in itself presents various pictures depending on the state of the cells and their histological treatment. Epicytes can be demonstrated in mammalian lungs with all good staining technics. In the mouse their structure and environment were found to be basically the same as in the nine other mammals investigated (Macklin, 1946 (12)).

Habitat

The mouse has the smallest alveoli of 10 mammals studied by Hartroft (1947 (6)), and this circumstance seems to influence the location of the partitional epicytes in that they are commonly found at the junctional points of the interalveolar septa, as Sjöstrand and Sjöstrand (1938 (23)) and others showed, rather than in the intervening parts of the walls, as often occurs in animals with larger alveoli (Macklin, 1946 (12)). Alveoli are boxlike cavities with one side, known as the mouth, open to the central space of an alveolar sac or duct (Hartroft, 1945 (5)). Opposite this mouth is the "base" functioning as a partition between alveoli on either side. It typically has three triangular segments sloping down to merge in a triradiate furrow, distinct only in well distended areas of lung. Each of these segments separates the contiguous parts of two alveoli. Thus any given alveolus may be in contact through its base with some three other alveoli. In these basal segments there are openings or pores, which often provide communications between adjoining alveoli; and when they do not do so it is because they are filled with parts of the bodies of epicytes or, it may be, dust cells (Macklin, 1948 (15)). An epicyte may be so sectioned as to show a part of itself in each of three such pores. By plugging these pores such an epicyte may have a share in the formation of the walls of as many as three neighboring alveolar spaces. Looked at in all three dimensions, it might be possible for an epicyte

to have four air faces, each belonging to a separate alveolus. The parts of the epicyte that project through these pores are known as processes or extensions. Usually these parts are unequal; often they are slender, but sometimes they are thick.

Material and Method

Lung material from over 40 healthy well grown albino white mice was studied. Various fixing fluids, notably Regaud's, and 10% neutral formalin, were applied in different ways. The small lungs of the mouse can be easily filled to moderate distention via the bronchial tree. The animal is etherized, the abdomen opened, and the great vessels cut. The diaphragm is nicked to collapse the almost blood-free lungs, and these are immediately filled by a hypodermic syringe with the needle inserted in the trachea. There is virtually no resistance from the elastic tissue of the delicate lungs, so that the cells are not subjected to undue pressure, and hence are retained in an approximately normal condition. Filling is carried to the stage of moderate inflation, as judged by the size of the lungs in the thoracic cavity. The trachea is then tied and the lungs, heart, and great vessels immersed in fixative. After becoming stiff, the lungs are cut into pieces which remain immersed in the fixing fluid. Many different staining technics were used, on frozen and paraffin sections. A number of strips of serial sections were available.

In studying the cytoplasm and processes of the epicytes, instructive preparations were obtained by intensive staining of very thin paraffin sections with Regaud's modification of Heidenhain's iron haematoxylin method for mitochondria (Cowdry, 1943 (3, p. 170)). Block mordanting in 3% aqueous potassium bichromate and section mordanting in 5% iron alum were both prolonged for as much as seven days. Staining in dilute (0.1% to 0.01%) aqueous haematoxylin continued for three to seven days. Differentiation was in iron alum, 5% diminishing to 2%, or in a saturated solution of picric acid in distilled water. Orange G and fast green were among the satisfactory counterstains. The character and variability of the cytoplasmic and nuclear staining will be considered in the next section. In this iron haematoxylin material the cytoplasm between the vacuoloids stains more or less darkly (in contrast to its eosinophilic reaction with the haematoxylin and eosin stain), but the degree of intensity varies widely in different epicytes. Much difference is seen in the vacuoloidal picture; in some cells these bodies are predominantly small, while in fewer cells they are large; again, in numerous cells they are of different sizes. No two cells were exactly alike, many being compact and rounded, while others were extended to considerable length. Often one, and more seldom two processes, can be seen. Nuclear staining, too, shows great variety.

Visual Representations, and Discussion

To give a conception of the alveolar location and general appearance of the partitional epicytes and their processes we may consider the sample plot of moderately expanded mouse lung tissue shown in Fig. 1, which measures

12,250 sq.μ. The photomicrograph is from a 2μ paraffin section stained with iron haematoxylin and orange G. The alveolar outlines appear open, *o o*, or closed, *c o*, respectively as their alveoli have been cut lengthwise to show the mouth or crosswise to exclude the mouth and thus to present an unbroken wall in that plane (Hartroft and Macklin, 1944 (7)). These walls look thin and attenuated, because they have been pressed upon by the preserving fluid in the alveoli, and most of the blood has been drained from the capillaries. In the lower right is an alveolar duct *al d*. The epicytes *ep* and the epicytic processes *pr* are situated at points where three or even four sections of alveolar wall meet (*ep* 10, *pr* 4), or in the edges of the mouths of alveolar sacs and alveoli opening from the alveolar ducts (*ep* 3, *pr* 1). *Ep* 3 has a free air face at the bottom of a little bay to its left. Even intensive staining has not revealed all parts of air faces at this magnification.

Epicytes

Eleven epicytes appear in this typical area of Fig. 1. They show varying grades of size, staining intensity, and vacuoloid condition. Epicyte 6 is large, being 15μ in maximum length. Examples of even greater size have been noted in other regions. Epicytes 1 and 2 are small, but may represent tangential sections that do not reveal their major dimensions. Others of intermediate size are seen, as *ep* 4 and *ep* 7. Epicytes 4, 6, and 7 are very densely stained in both cytoplasm and nucleus, and their vacuoloids are small and of slight prominence. The cell membranes are comparatively smooth. The rounded or oval nuclei are opaque and do not show vacuoloid impressions. Other epicytes, as 3, 5, and 9, are lighter in staining. In them the cytoplasm is of a bluish gray tone, and the vacuoloids, larger and more obvious, occupy relatively more space. The surfaces of such cells, in contact with air, are often rough. Certain of these epicytes, as *ep* 4, show something of an extension or process. In many of these cells, as *ep* 11, areas of both denser and lighter cytoplasm are seen.

Epicytic Processes

Four unnucleated vacuoloided structures with cloudy bluish cytoplasm in Fig. 1 are interpreted as epicytic processes cut transversely or obliquely. Each is definitely circumscribed by a thin cell membrane. Some lie in grooves in the alveolar wall, and others are completely surrounded by wall tissue. Processes 2, 3, and 4 are of relatively small size and may be assumed to have been sectioned at considerable distances from the main parts of their cells. They could hardly have been revealed without densification of their substance. In ordinary sections they would pass unnoticed. *Pr* 1 is larger and doubtless was cut near the nucleus of its cell.

Here and there are small obscure fragments that are provisionally taken to be detached epicytic cytoplasm (*d e c* 1 to 5). These minute fluffy indefinite shreds are of grayish shade with a tinge of blue, and lie upon the wall of the air space like a distant puff of smoke on the horizon. Some are only about 2μ across. Clearer areas in them, of hazy outline, are doubtless the remains of

vacuoloids or the recesses that contained them. In other regions an occasional shred like this has been seen in an air space, quite apart from the alveolar wall, having probably been buoyed up by the fixing fluid. The contour is not sharp, as is that of the epicytic processes. If these remnants are here correctly interpreted, the epicytes may be thought of as releasing parts of their substance on occasion, which suggests, perhaps, an apocrine type of secretion. I have not noted dust particles in these wisps. In places it is difficult or impossible to determine whether they are actually isolated, or are much attenuated extensions of epicytes or foam cells.

High Power Drawings and Photomicrographs

Epicyte 10 of Fig. 1 is interpreted in a drawing at higher magnification in Fig. 10. It is lodged in a crotch formed by three approximated alveolar walls. Its surface has two types of contact: (1) with the connective tissue of the alveolar walls, and (2) with the air of the alveoli. Each of these contact modes is represented by three areas. For the alveolar walls the surfaces are located above, below, and to the right. The relation of the cell membrane to this connective tissue is very intimate, but there is nothing here that can be regarded as a definitive basement membrane. For the air contacts there are three air faces—above, below, and to the left. Each of these bulges into its particular air space. The face to the left is roughened and suggests that vacuoloids are bursting through it like bubbles. This is an example of an epicyte with a thick process, which projects upward, filling a wide pore. It is full of vacuoloids, and looks like foam. The central nucleated region of the cell occupies an even larger pore. The vacuoloids are numerous, and suggest empty cavities because they have not taken the iron haematoxylin. In many, a minute granule at the center can be seen. That they are of firm consistency is indicated by the fact that those lying against the nucleus indent it, imparting to it a spiculated outline (Macklin, 1949 (19)). The nucleus is elongated, densely stained, and comes close to the cell membrane above.

Airfaciality

Sectional views of other trifacial epicytes of the partitional type are seen in Figs. 4, 6, 11, and 12. Many epicytes show only two air faces in the plane in which they happen to have been cut, as Figs. 2, 13, 15, 16, and 21; and others show but one, as Figs. 14, 17, and 20. Again, as many as four different air faces may be seen, as in Fig. 3. Additional air faces would doubtless be revealed in many of these cells if we could examine them in all three dimensions of space. Fig. 14 is a special case where the section shows a cell apparently almost completely enclosed by connective tissue, whereas in parts not included in the section it may be exposed to the air at other faces. This prospect is commonly encountered. An epicyte may misleadingly appear to be completely buried in connective tissue. Again, there may be only a narrow process, suggesting the Schnorkel of a U-boat, to bring it into apposition with the air. In this group of cells, inspection at once reveals the wide differences

in area of the air faces. Often an epicyte will present a broad lateral surface exposed to air (Figs. 2, 11, 15, 17, and 21) while other air faces will be relatively restricted.

Polarity: Parts: Processes

Epicytes develop from polarized epithelium (Macklin, 1946 (12)) and in certain sections a cell is cut approximately along the central axis as in Figs. 3, 6, 12, 13, 14, and 21, to suggest that it has retained this polarity in a demonstrable way. Then there are revealed (Figs. 3, 12, 13, 21) a broad and usually bulging head *h*, a circumscribed foot *f*, and a process, shank, or trunk *t* connecting the foot with the body of the cell. The foot may protrude (Figs. 13, 14, 21) or be situated in a depression (Fig. 12). Sometimes small spicules like stunted hairs project from the foot (Figs. 13, 14, and 15). These are best seen after intensive staining with iron haematoxylin. It has been suggested that epicytes are homologous with the ciliated cells of the bronchiolar epithelium (Macklin, 1949 (16); 1949 (18)), so that it would not be surprising if some of them produced rudimentary cilia. The establishment of this point would fortify the idea of polarity in the epicyte and distinguish the end that was originally surface from the end that was originally base. In most sections of epicytes such a spiculated extremity is not revealed. When the foot is very narrow, two parallel and closely approximated rods (Fig. 15) may be present here. Quite different are the plump processes (Fig. 10) or the rarified rough edged processes (Fig. 16). The head part *h* may comprise a large area (Figs. 6, 11, 13), a small area (Fig. 12), or one of intermediate type (Figs. 2, 3, 16). In plane views the epicyte may show one process (Figs. 3, 6, 12, 13, 14, 21) or two (Figs. 9, 11). The processes may be short (Fig. 6), long (Figs. 3, 12, 14), or intermediate (Figs. 11, 13). Sometimes the epicyte may be resolved mainly into processes (Fig. 11).

Processes are found sectioned at various slants. Those cut approximately longitudinally are represented by Figs. 3, 6, 12, 13, 14, and 21. What are taken to be cross sections are seen in Figs. 18, 19, 22, and 25. Figs. 23 and 24 probably represent oblique sections of processes. A process may be cut so as to appear completely enveloped by connective tissue of the alveolar wall (Figs. 18, 19, 22), or it may show one side free to the air while the other occupies a groove in the alveolar wall (Figs. 23, 24, 25).

Marginal Epicytes

These often occur singly, as that shown in Fig. 20, which is set in the connective tissue of the pleura like a tooth in a socket. It is without a process. Again, in Fig. 21 a marginal epicyte is shown which is set in the region of origin of an interalveolar partition. The process penetrates through this, terminating in a slightly protruding foot. Marginal epicytes do not differ essentially from those of the partitional type, but have not departed so widely from the fundamental epithelial morphology as have those whose base (head) has exchanged a connective tissue contact for an aerial one.

Nuclei

There is a wide variety of shape, ratio of area to that of cytoplasm, and staining intensity. Relatively large nuclei of intense staining and smooth contour (Fig. 13) seem to favor the epicytes whose cytoplasm is also darkly stained with iron haematoxylin and whose vacuoloids, though numerous, are small and relatively inconspicuous. It is possible that these may be less mature cells. Some nuclei are flattened (Fig. 10), or drawn out into a cylinder (Fig. 17). In the contours of many the impressions of vacuoloids are striking (Figs. 10, 11, 17, 20, and 21). Some nuclei have areas, often large, which do not take the iron haematoxylin; indeed many cases have been found where this staining was limited to a mere shell (Fig. 20). This nuclear achromasia seemed to be associated with a lightly staining cytoplasm containing large vacuoloids.

Extended Epicytes

Here and there in the sections are often found epicytes whose great length and narrowness (Figs. 8, 17) is notable. These usually show wide clublike ends and a central thin part where the nucleus, often in great measure achromatic, is sometimes so attenuated as to suggest a test tube. These long cells occur typically in the spiral wall formations delimiting the alveolated ducts. Vacuoloids occur in the dilated ends and often push their way into the nuclear contour (Fig. 17). Somewhat analogous hourglass shapes have been noted. The significance of these peculiar cell forms is obscure. They do not seem to represent stages of amitosis or mitosis.

Phagocytosis

Phagocytic activity has been reported for epicytes (Macklin, 1946 (12) *et al.*), and it is therefore of note that phagocytized material was not found in these cells in the apparently healthy mouse lungs here studied. Loosli (in Maximow and Bloom, 1948, p. 470 (21)) observed no phagocytic properties in attached septal cells (epicytes) even in acute pneumococcal infections of the lungs of dogs and monkeys. The function of epicytes under healthful routine conditions would seem, in the main at least, to be other than phagocytosis. The dust cells, on the other hand, show a widely different external and internal morphology and tinctorial reactivity, and are phagocytes *par excellence.* That foam and dust cells are extremes of oppositely directed differentiation processes that commence in the same mother cell, the epicyte, has been suggested (Macklin, 1949 (20)).

Rough Air Surfaces

Irregularity of air face has been noted (Figs. 10, 11, 16, 17, 20). Reference has been made to detached fragments that seem to be degenerate epicytic cytoplasm, lying free in the alveoli. Objects that seem to be free vacuoloids have been found in alveoli. Evidence like this suggests that epicytes may be of an exocrine glandular nature, liberating parts of their cytoplasm to subserve

some function, as in the maintenance of an optimum surface tension on the alveolar walls (Macklin, 1946 (12)).

Metabolic and Endocrine Functions?

Macklin (1946 (12)) and others have called attention to the close relationship that epicytes show to their capillaries, a relationship that suggests metabolic or endocrine functions for the collective "epicyte organ". Sjöstrand and Sjöstrand (1938 (23)) and Hayek (1942 (8)) are among those who have considered these possibilities.

Free Epicytes; Foam Cells

In sections one sometimes encounters foam cells with many of the attributes of epicytes, but which are not locked in pores, but rather are free within the alveoli (Figs. 5, 7). These are sometimes of relatively simple form, as in Fig. 5, where the large vacuoloids are mainly in but one layer. The nucleus is made irregular by the proximity of vacuoloids. Other foam cells are larger and crowded with well developed vacuoloids (Fig. 7). Great lightly staining foam cells have been recovered by the gash-irrigation method (Macklin, 1947 (14); 1949 (20)); but it is remarkable how seldom these are obtained in this way in comparison with the dust cells, which emerge in troops. From the literature and from experiments there is reason to believe that certain epicytes release their holds upon the alveolar walls to become free foam cells. In the vacuoloids of such foam cells, and indeed in those of many epicytes, a very fine central granule, already mentioned in epicytes, may sometimes be seen just on the limit of visibility. The mode of nutrition of these free foam cells, in the gaseous *milieu*, is obscure, as it is for their companions the dust cells.

Other Stainings

The details of epicytes, particularly as related to the processes, were best revealed by the intensive use of the iron haematoxylin technic, and they have been illustrated by a typical case, mouse No. 30 of Series 93. The various other stainings available afforded valuable supplementary evidence in confirmation of the conclusions reached. They also revealed other features of the epicytes and foam cells, which it is hoped will be reported later.

Acknowledgments

The writer wishes to thank the National Research Council of Canada for aiding this research project with a grant for technical assistance, and Mr. Charles E. Jarvis for his excellent work in the preparation of the material and photomicrographs.

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Explanation of Figures on following page.

Explanation of Figures

All figures on Plate I are photomicrographs of mouse epicytes; Figs. 10–25 are pen and ink drawings by camera lucida of mouse epicytes or their processes.

Sections of origin: all figures are from Series 93, Mouse 30. Fig. 3 is from Sec. 8; Figs. 5, 11, 12—Sec. 55; Figs. 8, 9—Sec. 57; Fig. 14—Serial Sec. 6, Row 2, Slide 3; Fig. 15—Serial Sec. 4, Row 2, Slide 3. The rest of the figures are from Sec. 47.

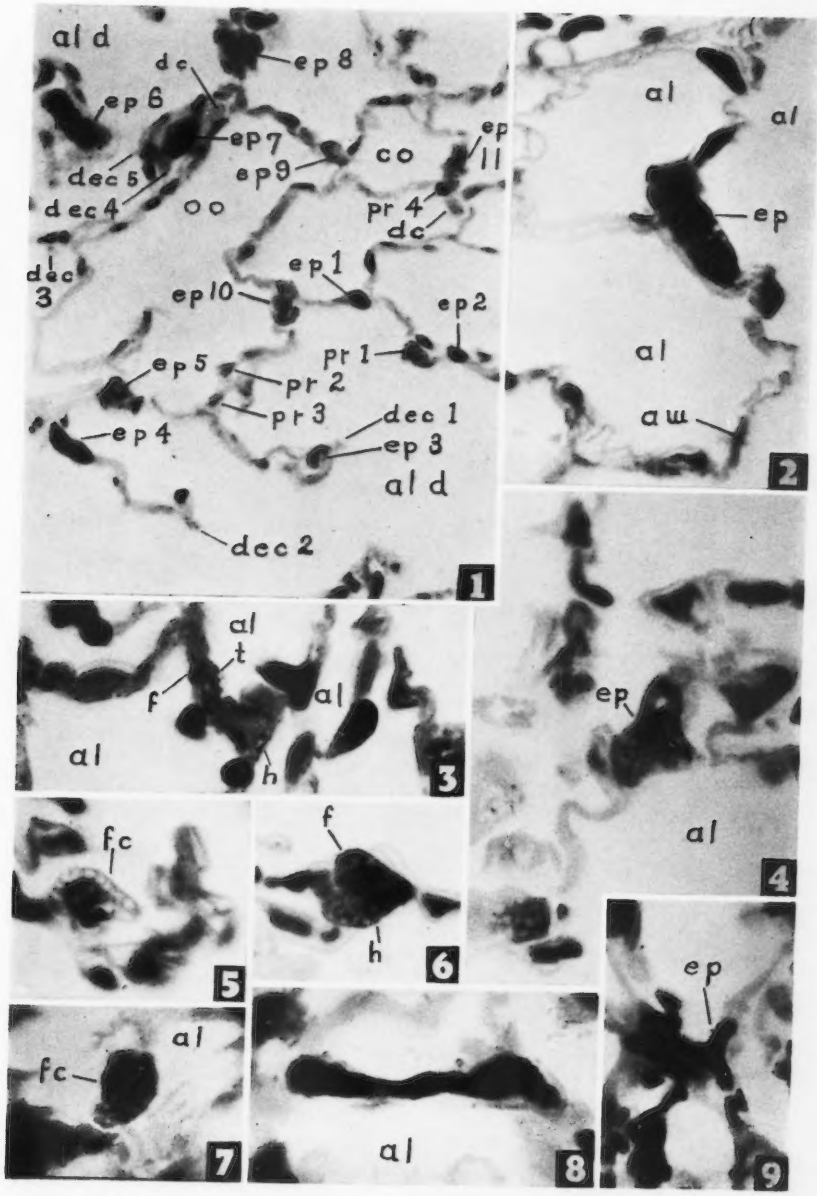
Thicknesses of sections: Figs. 14, 15, 3μ; Figs. 8, 9—flattened frozen, 30μ; all other figures, 2μ.

Stainings: *Fig. 3—Altmann, for mitochondria.*
Figs. 5, 11, 12—iron haematoxylin and fast green.
Figs. 14, 15—iron haematoxylin and aurantia.
All other figures—iron haematoxylin and orange G.

Magnifications: *Fig. 1—×700.*
Figs. 2, 3, 4—×1663.
Figs. 5, 6, 7, 8—×1488.
Fig. 9—745.
Figs. 10 to 25—×2100.

Abbreviations:

al	*—alveolus*
al d	*—alveolar duct*
a w	*—alveolar wall*
c o	*—closed outline of alveolar wall*
d c	*—dust cell*
d e c	*—detached epicytic cytoplasm*
ep	*—epicyte*
f	*—foot of epicyte*
f c	*—foam cell*
h	*—head of epicyte*
o o	*—open outline of alveolar wall*
pr	*—process of epicyte*
t	*—trunk of epicyte*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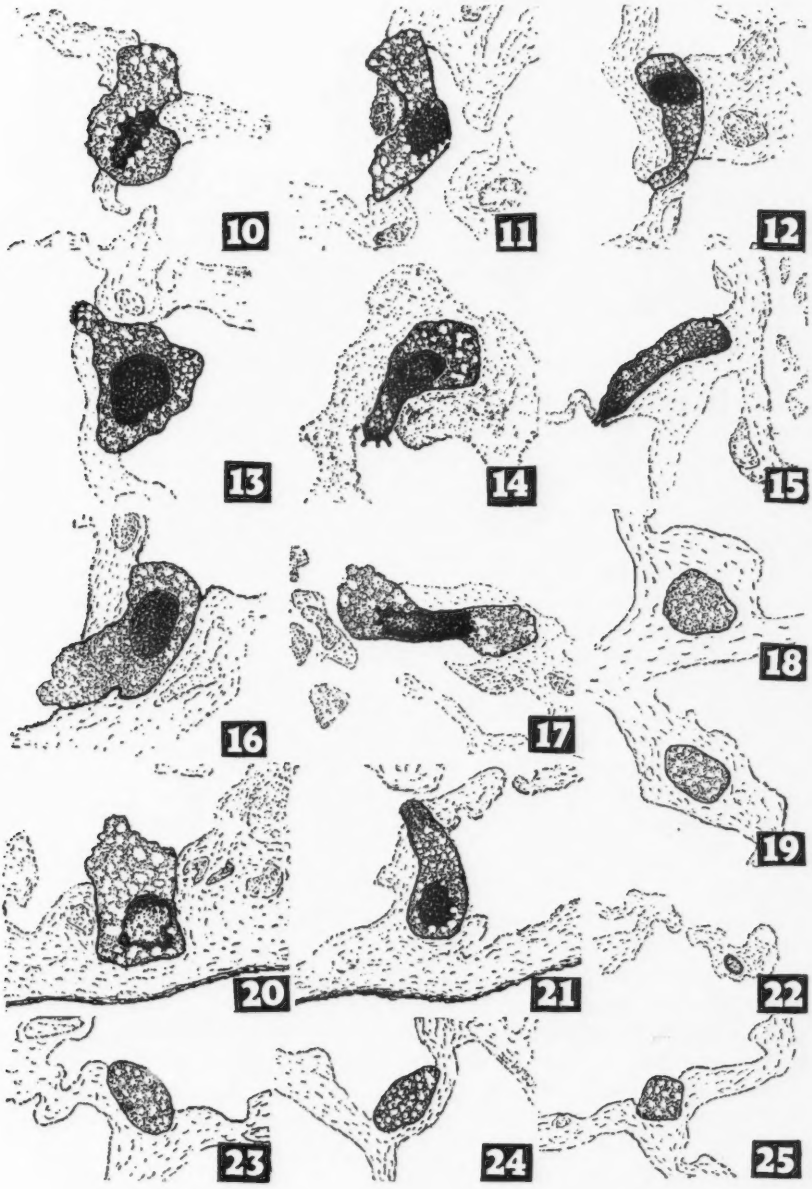
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A STUDY OF SIZE INHERITANCE IN THE HOUSE MOUSE

I. THE EFFECT OF MILK SOURCE[1]

By L. Butler[2] and J. D. Metrakos[3]

Abstract

Three strains of mice were used to study the effect of fostering on the growth pattern of the mouse. The strains used breed true for size and have been designated as "Large", "Small", and "Intermediate". The 14-day mean weight of mice that received milk from "Large" strain mothers is significantly different from those that received milk from either the "Small" or the "Intermediate" strain mothers. Although these differences tend to remain, they are not statistically significant at 140 days. The significance of these results are discussed in relation to the arithmetic and geometric concepts of polygenic growth.

Introduction

An investigation of size inheritance was begun in April 1947 utilizing the large and small strains of mice developed by MacArthur (6). Crosses were made between the large and small strains and also between these strains and an intermediate or "normal-sized" mouse. Growth studies of these hybrids indicated that more experimental work was needed to distinguish between the relative influences of the genetic and environmental factors that interact to bring about final body size. In the early stages of postnatal growth two of the most important factors influencing growth are litter size, and the quantity and quality of the mother's milk. This experiment is concerned chiefly with the latter factor and was designed to investigate the question whether the differences in the adult weight between mice of distinct size were due entirely to differences in the genetic potential or partly to differences in the early milk supply of the suckling young. Furthermore, the observations were continued far beyond puberty in order to determine whether any differences resulting from fostering would be maintained in the adult mouse. The data on litter size will be discussed in a subsequent paper.

Description of Experimental Stocks

The large and small strains used in this experiment have been produced by about 20 generations of mass selection by MacArthur (8). In general, inbreeding was avoided in order to keep the gene pool as large as possible; but some inbreeding may have occurred as concomitant with selection. After the large and small stocks were brought to McGill, some inbreeding was necessary because of the restricted number of breeders available. Under these conditions the extremes of body size reached by mass selection were not maintained. Nevertheless the large and small races remained significantly

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different from the intermediate or "normal-sized" A-strain mouse that was used in this experiment (See Tables I and II).

In this experiment the large race males at 60 days were characterized by a mean body weight of 39.62 gm. with a standard error of ± 0.89 gm. This is 17.50 gm. or 79.1% larger than that of the A-strain males, which are not selected for body size. (The comparable figures for the females are 32.01 $\pm$ 0.68 gm. or 69.8% larger than the A-strain.) The average litter size is 10.31 $\pm$ 0.35 as compared with a litter size of 6.71 $\pm$ 0.24 for the A-strain. The large mice are lethargic and have a coat color that is usually dilute brown or dilute brown agouti. The small race males are characterized by a body weight of 17.47 $\pm$ 0.62 gm., which is 21.0% smaller than the A-strain males. (The comparable figures for the females are 13.81 $\pm$ 0.62 gm. or 26.7% smaller than the A-strain.) They are very active as compared with the large strain, have a litter size of 6.04 $\pm$ 0.20, and are usually black in color.

The A-strain, which was used in the fostering experiments (but not in the hybrid crosses) was originated by Strong in 1921, and has been inbred by brother–sister matings for over 200 generations. Its genotype is ccbbaa. The average male weighs at 60 days 22.12 $\pm$ 0.53 gm., and the average female 18.85 $\pm$ 0.48 gm. The litter size is 6.71 $\pm$ 0.24.

For simplicity and convenience the strains will be indicated by the single letters L, S, and A, for large, small, and A-strain respectively. The milk source will be designated by the denominator of the fraction so that "A/S male" means an A-strain male fostered on a small strain female. Likewise "L/L female" signifies a female of the large strain nursed by its own mother. Age will be represented by the letter D followed by a number denoting the number of days after birth.

Outline of the Fostering Experiment

All mice used in this experiment were kept in the same room and fed a standard diet of fox-chow supplemented once a week by lettuce leaves, and milk-soaked bread. The breeding cages were checked thrice weekly and the pregnant females isolated shortly before term. The pregnancy cages were checked daily and all litters that had been born during the previous 24 hr. were split up into groups of four. Each group consisted of two males and two females. The groups were then either placed with a foster mother or left with their own mother depending on the number and nature of the litters born on that day. Foster cages were made up only when the mother and the foster mother gave birth to their litters on the same day. No difficulty was encountered in getting the foster mother to accept the new litter. At first, the litter to be fostered would be rubbed for a few minutes with some of the soiled sawdust from the foster mother cage. Later this procedure was discontinued for it was found unnecessary. Out of 51 litters that were fostered, only three had to be discarded because one or more of the four mice failed to reach 20 days of age.

As the pregnancy cages were examined every morning, and as most births occur between 12 to 4 A.M. (12), young that were fostered had not received more than a few hours of milk from their true mothers. Mice that could not be used to form a brother–sister group of four were discarded. Groups in which one or more members failed to reach D-20 were also discarded as mentioned above.

Up to D-60 the mice were weighed every two days. From D-60 to D-140 they were weighed every four days. Thus 51 weights were recorded for each mouse in the period of 140 days. Weighings were made to the nearest decigram on a Harvard Trip Balance.

At D-30 when the mice were pedigreed, they were isolated according to their sex, strain, and milk source. No more than six mice were placed in each cage. At this time the mice were also etherized to the point of complete relaxation and were stretched out on a centimeter ruler. The length from the tip of the snout to the anus was recorded as the "body" length, and from the snout to the tip of the tail as the "overall" length. The "body" and "overall" lengths were taken again at D-60, D-90, and D-120 employing the same technique.

At D-140 the male mice that were not used as breeders were killed by etherization and the right hind femur and tibia dissected. The length of each of these two bones was measured with micrometer calipers calibrated to read to 0.01 mm. The distance from the greater trochanter to the articular sesamoids was taken as the length of the femur; and from the lateral tuberosity to the lateral malleolus as the length of the tibia.

Effects of Fostering on Body Weight

In view of the lack of agreement on the statistical treatment of growth data and also on the use of the lactation-efficiency index (13), it was found desirable to use a new technique of evaluating the causes of weight differences. If the conventional growth curves for L/L, L/S, S/L, and S/S males are plotted as in Fig. 2, some comparisons can be made between the nonfostered L and S and the reciprocal fosterings between these strains. This graphical representation shows that L/S males grow more slowly up to D-16 and then grow faster than L/L males. This continues until D-36 after which the growth rates of fostered and nonfostered mice are almost identical so that differences present on D-36 tend to be maintained to maturity. The same relationship exists between L/S and L/L females. Similarly, S/L mice grow faster than S/S mice of the same sex up to D-14, and from then to D-36 more slowly than S/S. After D-36 the growth rates of fostered and nonfostered are almost identical.

It is interesting to note the situation that arises between D-6 and D-16 (See Fig. 3). During this period in both sexes the S/L mice are larger than the L/S. At D-14, $P = .02$ for females and .01 for males. At this time,

therefore, the effect of milk source on size appears to overshadow that of the genetic potential. However, when both L and S mice are exposed to the same milk source, the genetic potentialities again seem to show up, since L/A are

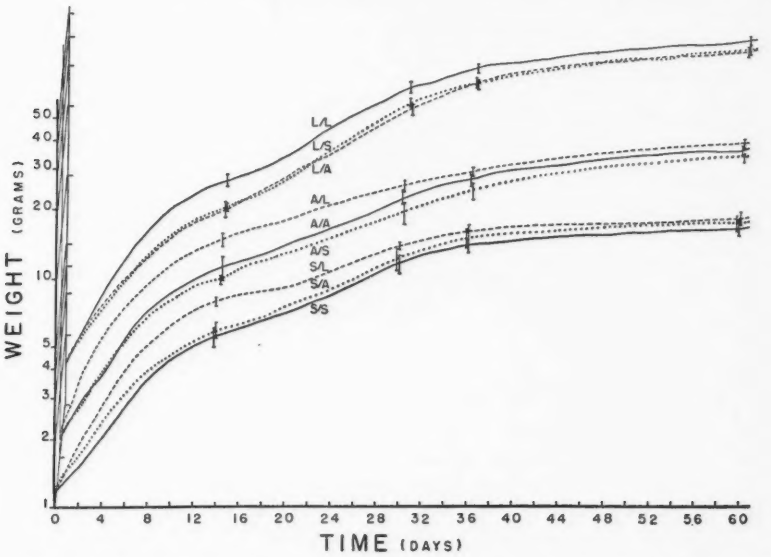

FIG. 1. *Logarithmic growth curve of S (outer scale), A (middle scale), and L (inner scale) nonfostered and fostered male mice. ± 2 S.E. are shown at D-14, D-30, D-36, and D-60.*

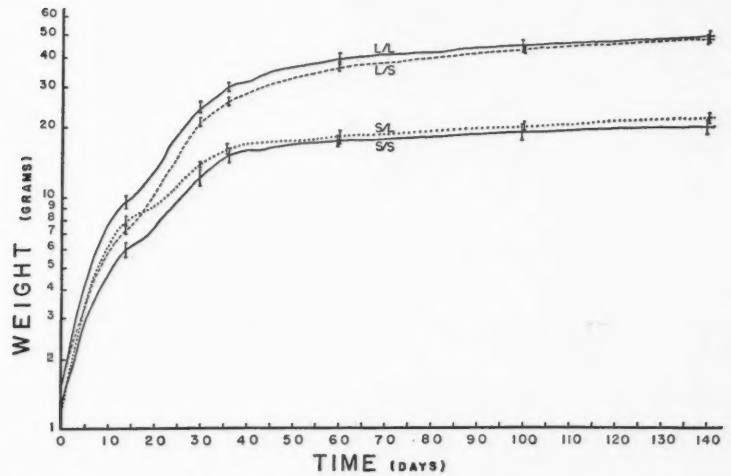

FIG. 2. *Weight difference due to reciprocal fostering between S and L male mice. ± 2 S.E. are shown at D-14, D-30, D-36, D-60, D-100, and D-140.*

larger than S/A (See Table I and Fig. 1). The role of the genetic potential is, however, a complicated one and may include for instance the influence of a stronger suckling stimulus provided by the L young to the A mother, which

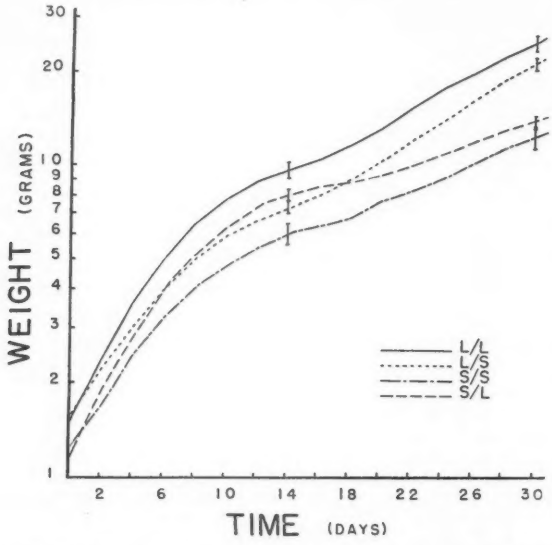

FIG. 3. *Weight differences due to reciprocal fostering between S and L male mice.* ± 2 *S.E. are shown at D-14 and D-30.*

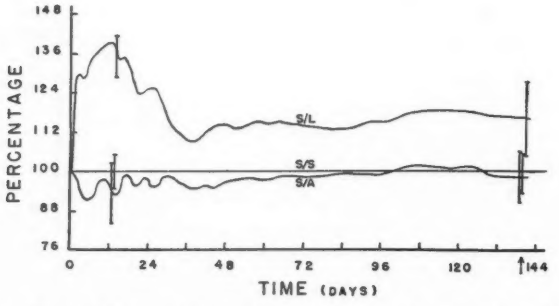

FIG. 4. *Weight differences (expressed as a percentage of the nonfostered) due to fostering female S mice.* ± 2 *S.E. are shown at D-14 and D-140.*

may increase their milk supply. Again, the fact that the lethargic L young have to cope with an active S foster mother, may explain why the L/S are smaller than the S/L during the height of the nursing period.

The differences due to fostering may be emphasized by the following method. If the nonfostered mice of each strain are considered as the normal or control groups, then their weight throughout the time scale may be represented as a

TABLE I

MEAN WEIGHT IN GM. (± S.E.) OF NONFOSTERED AND FOSTERED MICE

Sex	Day	Small			A-strain			Large		
		S/S	S/A	S/L	A/S	A/A	A/L	L/S	L/A	L/L
Female	0	1.08 ± 0.03	1.08 ± 0.05	1.10 ± 0.03	1.17 ± 0.03	1.18 ± 0.02	1.25 ± 0.05	1.51 ± 0.04	1.46 ± 0.02	1.48 ± 0.03
	14	5.87 ± 0.17	5.48 ± 0.26	7.91 ± 0.20	6.39 ± 0.16	6.70 ± 0.16	8.25 ± 0.26	7.26 ± 0.19	6.77 ± 0.28	9.64 ± 0.29
	30	10.58 ± 0.59	10.38 ± 0.38	12.26 ± 0.41	11.39 ± 0.47	12.08 ± 0.33	13.96 ± 0.33	20.07 ± 0.59	16.83 ± 0.87	22.47 ± 0.58
	36	12.73 ± 0.61	12.25 ± 0.41	13.95 ± 0.54	14.01 ± 0.52	14.56 ± 0.32	15.98 ± 0.33	23.51 ± 0.58	22.38 ± 0.73	26.48 ± 0.52
	60	13.81 ± 0.62	13.51 ± 0.60	15.88 ± 0.79	18.87 ± 0.41	18.85 ± 0.48	20.45 ± 0.21	29.74 ± 0.52	30.25 ± 0.69	32.01 ± 0.68
	100	14.75 ± 0.57	14.85 ± 0.63	17.17 ± 0.81	21.30 ± 0.44	20.95 ± 0.33	22.74 ± 0.32	34.63 ± 0.71	34.88 ± 0.90	36.86 ± 0.68
	140	15.77 ± 0.55	15.50 ± 0.65	18.47 ± 0.85	22.49 ± 0.41	22.66 ± 0.38	24.48 ± 0.42	37.31 ± 0.80	36.77 ± 1.20	39.34 ± 0.80
	N	12 - 11	13	11	15	10	11	22	12	18
Male	0	1.26 ± 0.04	1.19 ± 0.07	1.19 ± 0.03	1.24 ± 0.35	1.23 ± 0.03	1.36 ± 0.05	1.56 ± 0.04	1.56 ± 0.03	1.55 ± 0.03
	14	6.01 ± 0.24	5.63 ± 0.27	8.01 ± 0.19	5.92 ± 0.18	6.85 ± 0.38	8.99 ± 0.27	7.32 ± 0.16	7.26 ± 0.27	9.64 ± 0.32
	30	12.25 ± 0.59	11.63 ± 0.51	13.87 ± 0.05	11.77 ± 0.61	13.72 ± 0.43	15.51 ± 0.33	21.21 ± 0.53	19.57 ± 0.69	24.72 ± 0.71
	36	15.04 ± 0.64	14.10 ± 0.52	16.13 ± 0.49	14.84 ± 0.67	16.65 ± 0.38	17.93 ± 0.35	26.04 ± 0.55	25.91 ± 0.50	30.17 ± 0.71
	60	17.47 ± 0.62	16.34 ± 0.88	18.24 ± 0.63	20.65 ± 0.43	22.12 ± 0.53	23.62 ± 0.68	36.52 ± 0.48	36.00 ± 1.23	39.62 ± 0.89
	100	19.19 ± 0.82	18.23 ± 0.91	20.38 ± 0.69	23.69 ± 0.41	25.31 ± 0.74	26.50 ± 0.63	43.50 ± 0.68	44.92 ± 2.12	45.14 ± 1.30
	140	20.10 ± 0.82	18.39 ± 1.02	21.80 ± 0.67	25.73 ± 0.43	27.26 ± 0.79	28.84 ± 0.75	47.10 ± 0.90	48.70 ± 2.63	48.59 ± 1.40
	N	14	11 - 10	18	17	10	14	28	10	18

straight line. The weights of the fostered mice may then be represented as a percentage of the nonfostered weights. In this way the cumulative size effects produced by fostering can be readily observed as in Figs. 4 to 9 inclusive.

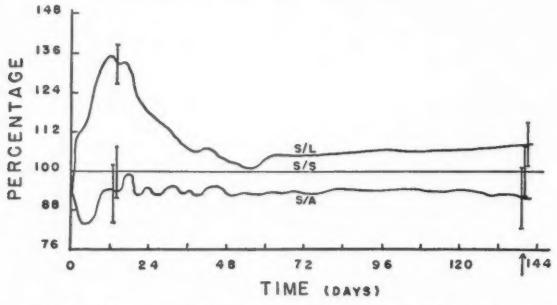

FIG. 5. *Weight differences (expressed as a percentage of the nonfostered) due to fostering male S mice. ± 2 S.E. are shown at D-14 and D-140.*

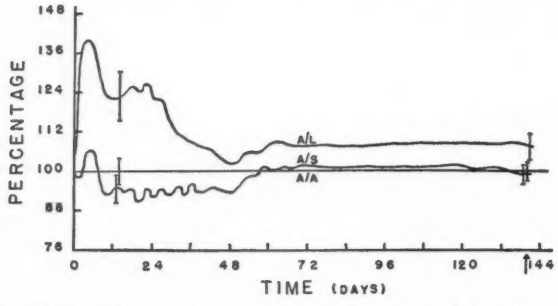

FIG. 6. *Weight differences (expressed as a percentage of the nonfostered) due to fostering female A mice. ± 2 S.E. are shown at D-14 and D-140.*

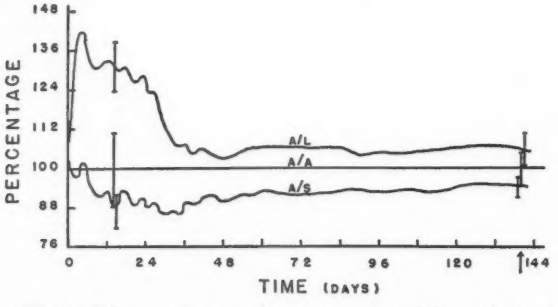

FIG. 7. *Weight differences (expressed as a percentage of the nonfostered) due to fostering male A mice. ± 2 S.E. are shown at D-14 and D-140.*

The effect of fostering S mice on L mothers was to increase the weight of the S strain. This increase reached its maximum at D-12 when the males were 35.8% and the females 39.4% larger than the normals. From this peak

the increase that resulted from fostering fell off until about D-36, which is the point of inflection of the growth curve. After this drop towards the base line, the increase that can be attributed to fostering stays constant for the life

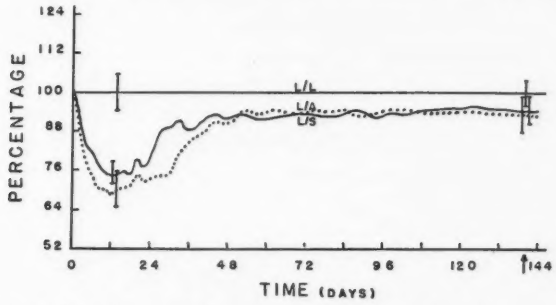

FIG. 8. *Weight differences (expressed as a percentage of the nonfostered) due to fostering female L mice. ± 2 S.E. are shown at D-14 and D-140.*

of the mouse, and may even increase slightly. The differences between fostered and nonfostered mice are not significant after D-30, but since they are always in the same direction they are real differences and the milk source may be responsible for as much as 15 to 18% increase in the mature weight. The females appear to retain twice the amount of gain over the nonfostered as do the males. The percentages are 117.1% for females and 108.5% for males.

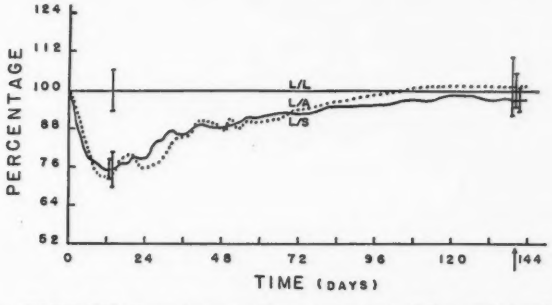

FIG. 9. *Weight differences (expressed as a percentage of the nonfostered) due to fostering male L mice. ± 2 S.E. are shown at D-14 and D-140.*

The effect of fostering S and A was to decrease the mean weight of the S strain. The maximum decrease occurred on D-4 after which the females returned to normal, but the males remained about 6% below the normal. With the exception of the D-4 differences, the decreases are not significant. The marked differences at D-4 may be due to the initial psychological effects of fostering per se. (The "psychological" effects may include such influences as odor recognition, anatomical incompatibility as between size of foster mother's nipples and size of fostered young's mouth, etc. These influences may be assayed in part by fostering mice on a foster mother of the same strain.)

When A is fostered on L there is a maximum increase of 40.4% for females and 41.8% for males, which is reached at D-4, and then the percentage increase of A/L over A/A drops gradually to D-36 after which it is stabilized at a little over 6% for both sexes. The fostering of A on S decreased the size of the A mice. This decrease was more or less progressive, reaching its maximum between D-20 and D-30. There was a significant difference in the response of the two sexes to fostering. The females showed only 6% retardation and by D-60 were back to normal. The males on the other hand were retarded 14% and never completely recovered from this early setback. At D-140 they were still 6% below normal. This difference is not statistically significant from A/A ($P = .123$) but is significantly different from the A/L mean weight ($P = .003$).

The fostering of L on either S or A mothers led to a decrease in weight. This reached its maximum effect on D-12 and thereafter the fostered mice began to approach the nonfostered ones in size. The males showed less permanent effects than the females, in fact the L/A (48.70 ± 2.63) males were almost identical with the L/L (48.59 ± 1.40) ones at D-140.

To test the statistical significance of the above differences Student's t test was used. The results of these tests are summarized in Table II, and show that the following differences are significant:—

1. Both male and female mice that were fostered on L showed highly significant differences ($P = .001$) at D-14 over the nonfostered ones. In the case of the females these differences tend to remain significant at the 5% level right up to D-140. In the males, however, the significance of the differences has disappeared in all cases by D-60.

2. In general, when the test of significance is made between mice that received the S type of milk and similar mice that received the A type, no marked differences were observed. This is well illustrated in Figs. 4 to 9 where the lines X/S follow closely the X/A ones.

3. The fostering of S mice on A mothers had no significant effect; but when L mice were fostered on similar mothers, a significant decrease was produced, which remained until D-36. (This statement may be looked upon as a corollary of the above two statements.)

4. Male and female L/S remained significantly retarded until D-60, with the height of retardation at around D-14.

5. The A/S females showed no significant retardation, but the A/S males were significantly retarded at the 5% level up until D-60.

6. The test of significance between A/S and A/L males and females shows that a significant difference remains in the adult mice.

This last point strongly proves that early nutrition has a significant effect on mature size; the weight-increasing effects of L milk and the weight-decreasing effects of S milk being persistent. When the D-60 weights are taken as the criterion of body size, the varied milk sources can be expected to account

TABLE II

TEST OF SIGNIFICANCE OF WEIGHT DIFFERENCES

Sex	D.F.	Test	Day													
			0		14		30		36		60		100		140	
			d	P	d	P	d	P	d	P	d	P	d	P	d	P
Female	23 - 22	S/A-S/S	0.00	.900	−0.39	.300	−0.20	.800	−0.48	.600	−0.30	.800	+0.10	.500	−0.27†	.800
	21	S/L-S/S	+0.02	.700	+2.04	.000*	+1.68	.030*	+1.22	.200	+2.07	.050*	+2.42	.023*	+2.70	.014*
	22	S/L-S/A	+0.02	.800	+2.43	.000*	+1.88	.003*	+1.70	.019*	+2.37	.026*	+2.32	.034*	+2.97	.011*
	23	A/A-A/S	+0.01	.800	+0.31	.200	+0.69	.300	+0.55	.400	−0.02	.900	−0.35	.600	+0.17	.800
	24	A/L-A/S	+0.08	.200	+1.86	.000*	+2.60	.000*	+1.97	.004*	+1.58	.002*	+1.44	.015*	+1.99	.002*
	19	A/L-A/A	+0.07	.300	+1.55	.000*	+1.88	.000*	+1.42	.006*	+1.60	.006*	+1.79	.001*	+1.82	.005*
	32	L/A-L/S	−0.05	.300	−0.49	.200	−3.24	.004*	−1.13	.300	+0.51	.600	+0.25	.900	−0.54	.800
	38	L/L-L/S	−0.03	.600	+2.38	.000*	+2.40	.004*	+2.97	.000*	+2.27	.011*	+2.23	.023*	+2.03	.076
	28	L/L-L/A	+0.02	.600	+2.87	.000*	+5.64	.000*	+4.10	.000*	+1.76	.080	+1.98	.093	+2.57	.086
Male	23 - 22	S/A-S/S	−0.07	.400	−0.38	.800	−0.62	.500	−0.94	.300	−1.13†	.400	−0.96	.500	−1.71	.300
	30	S/L-S/S	−0.07	.200	+2.00	.000*	+1.32	.034*	+1.09	.200	+0.77	.400	+1.19	.300	+1.70	.200
	27 - 26	S/L-S/A	0.00	.900	+2.38	.000*	+2.24	.000*	+2.03	.008*	+1.90†	.090	+2.15	.072	+3.41	.011*
	25	A/A-A/S	−0.01	.900	+0.93	.037*	+1.95	.014*	+1.81	.028*	+1.47	.040*	+1.62	.068	+1.52	.200
	29	A/L-A/S	+0.12	.049*	+3.07	.000*	+3.74	.000*	+3.09	.000*	+2.97	.001*	+2.81	.001*	+3.10	.001*
	22	A/L-A/A	+0.13	.020*	+2.14	.000*	+1.79	.003*	+1.28	.023*	+1.50	.095	+1.19	.300	+1.58	.200
	36	L/A-L/S	0.00	.200	−0.06	.200	−1.64	.064	−0.13	.300	−0.52	.600	+1.42	.900	+1.60	.600
	44	L/L-L/S	−0.01	.900	+2.32	.000*	+3.51	.000*	+4.13	.000*	+3.10	.002*	+1.64	.300	+1.49	.400
	26	L/L-L/A	−0.01	.600	+2.38	.000*	+5.15	.000*	+4.26	.000*	+3.62	.081	+0.22	.092	−0.11	.800

NOTE: *P values less than 0.100 are given to the nearest thousandth; all other values to the nearest tenth.*

* *P values that are significant at the 5% level.*

† *From this day on the lesser D.F. was used.*

for the percentage differences indicated in Table III below. Note that the males show the greatest decrease from fostering on an inferior milk supply and the least increase from fostering on a superior milk supply.

TABLE III

PERCENTAGE DIFFERENCE BETWEEN NONFOSTERED AND FOSTERED MICE AT 60 DAYS

Sex	Small		A-strain		Large	
	S/A	S/L	A/S	A/L	L/S	L/A
♀♀	−2.2%	+15.0%	+0.1%	+8.5%	−7.1%	−5.5%
♂♂	−6.5%	+ 4.4%	−6.6%	+6.8%	−7.8%	−9.1%

The Effects of Fostering on Body and Overall Lengths

MacArthur (7) has shown that there is heterogonic growth in the various features of the mouse such as the tail, ear, feet, etc. Green and Fekete (4) have shown that the relationship between weight 'y' and length 'x' can be expressed by the equation $y = 0.000137\ x^{2.68}$. This being so, it is not to be expected that body length will show fostering differences of the same magnitude as those exhibited by weight. Furthermore, body length also shows heterogony with tail length so that the overall length differences may be significant at one age and not at another.

In Table IV the body and overall lengths of mice at D-30, D-60, D-90, and D-120 are compared and tested for significance by means of the "t" test. From this table it is evident that when S/L are compared with S/S a significant difference is found at D-30 and at D-60 in the females only. The overall length is significant in the females at D-60 and at D-90 but not in the males. These sex differences agree closely with the results obtained when the weights were compared. In the comparison of fostered and nonfostered L mice it is noted that body length differences are significant in the females only at D-30, while in the males they are highly significant at both D-30 and at D-60.

In general the results show that differences do exist at D-30 and some persist until D-60, but the tendency is for them to disappear in the older animal. The increase in weight due to fostering is not reflected in the linear measurements of the mature mouse. Measurements were not taken at D-14, but Figs. 10 and 11 show that differences in length exist at this time between fostered and nonfostered animals.

The Effect of Fostering on the Length of Femur and Tibia

The femur and tibia lengths for the relatively few animals used in this portion of the experiment do not show significant differences as the result

PLATE I

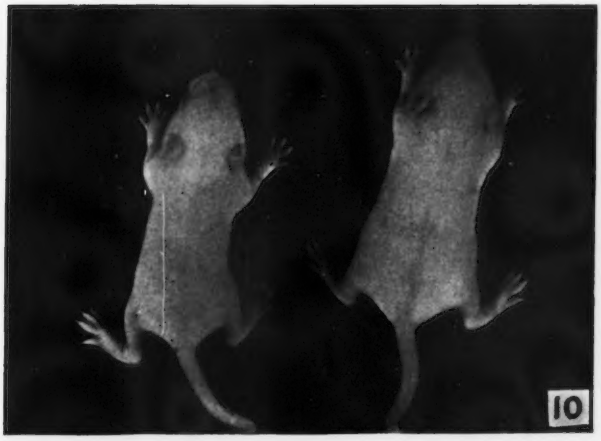

FIG. 10. *Female mice:— at D-14 left mouse (A/A) weighed 5.4 gm.; right mouse (A/L) weighed 7.3 gm. and is about 8% longer than the A/A mouse.*

FIG. 11. *Male mice:— at D-14 left mouse (A/A) weighed 5.9 gm.; right mouse (A/L) weighed 8.7 gm. and is about 10% longer than the A/A mouse.*

TABLE IV

TEST OF SIGNIFICANCE OF LENGTH DIFFERENCES

Sex	D.F.	Test	Day															
			30				60				90				120			
			Body		Overall		Body		Overall		Body		Overall		Body		Overal	
			d	P	d	P	d	P	d	P	d	P	d	P	d	P	d	P
Female	23	S/A–S/S	−0.17	.300	−0.58	.099	−0.22	.065	−0.41	.200	−0.19	.300	−0.19	.700	−0.15	.200	−0.14	.800
	21	S/L–S/S	+0.28	.032*	+0.62	.053	+0.46	.014	+1.00	.002*	+0.23	.300	+0.81	.022*	+0.16	.500	+0.71	.070
	22	S/L–S/A	+0.45	.007*	+1.20	.000*	+0.68	.002*	+1.41	.000*	+0.42	.041*	+1.00	.011*	+0.31	.200	+0.85	.049*
	23	A/A–A/S	+0.08	.700	+0.01	.900	−0.27	.010*	−0.26	.200	−0.23	.010*	−0.22	.064	−0.21	.012*	−0.14	.200
	24	A/L–A/S	+0.54	.002*	+1.28	.000*	−0.01	.900	+0.10	.600	0.00	.900	+0.30	.093	−0.01	.900	+0.36	.060
	19	A/L–A/A	+0.46	.002*	+1.27	.000*	+0.26	.023*	+0.36	.009*	+0.23	.088	+0.52	.011*	+0.20	.150	+0.50	.030*
	32	L/A–L/S	−0.36	.047*	−0.52	.300	−0.15	.200	−0.20	.400	−0.14	.300	−0.13	.600	−0.08	.600	−0.05	.900
	38	L/L–L/S	+0.32	.022*	+0.08	.900	+0.01	.900	−0.03	.900	+0.01	.500	+0.08	.700	+0.01	.900	+0.14	.600
	28	L/L–L/A	+0.69	.000*	+0.60	.200	+0.16	.300	+0.17	.500	+0.15	.300	+0.21	.400	+0.29	.600	+0.19	.500
Male	23–22	S/A–S/S	−0.12	.600	−0.71	.092	−0.32†	.080	−0.73†	.061	−0.25	.200	−0.55	.300	−0.16	.500	−0.54	.300
	30	S/L–S/S	+0.26	.200	+0.32	.400	+0.06	.700	+0.17	.600	+0.11	.500	+0.06	.900	+0.10	.600	+0.05	.900
	27–26	S/L–S/A	+0.38	.027*	+1.03	.001*	+0.90†	.017*	+0.36†	.008*	+0.36	.023*	+0.61	.200	+0.26	.200	+0.59	.200
	25	A/A–A/S	+0.28	.088	+0.07	.900	+0.08	.500	+0.14	.300	−0.09	.500	−0.10	.600	−0.10	.400	−0.15	.400
	29	A/L–A/S	+0.68	.000*	+1.24	.001*	+0.23	.000*	+0.28	.010*	+0.10	.300	+0.12	.500	+0.09	.500	+0.07	.700
	22	A/L–A/A	+0.40	.001*	+1.17	.000*	+0.15	.200	+0.14	.300	+0.19	.058	+0.22	.200	+0.19	.062	+0.22	.300
	36	L/A–L/S	−0.14	.500	+0.10	.900	+0.05	.600	+0.15	.300	−0.08	.600	+0.03	.900	−0.03	.900	+0.10	.800
	44	L/L–L/S	+0.42	.000*	+0.41	.300	+0.36	.000*	+0.28	.083	+0.08	.200	+0.17	.200	+0.06	.500	+0.01	.900
	26	L/L–L/A	+0.56	.007*	+0.31	.500	+0.31	.009*	+0.13	.600	+0.16	.300	+0.14	.600	+0.09	.600	−0.09	.800

NOTE: *P values less than 0.100 are given to the nearest thousandth; all other values to the nearest tenth.*

* *P values that are significant at the 5% level.*

† *From this day on the lesser D.F. was used.*

of fostering. It is noteworthy, however, that small differences do exist (Table V), and that these tend on the whole to be in the direction expected from the weight analyses.

TABLE V

MEAN LENGTH IN MM. (± S.E.) OF RIGHT FEMUR AND TIBIA OF MALE MICE

	N	Femur	Tibia
S/S	11	13.27 ± 0.35	16.06 ± 0.24
S/A	10	13.26 ± 0.45	15.79 ± 0.41
S/L	18	13.43 ± 0.22	16.20 ± 0.18
A/S	13	14.50 ± 0.23	17.29 ± 0.17
A/A	8	14.84 ± 0.28	17.47 ± 0.19
A/L	12	14.63 ± 0.17	17.54 ± 0.21
L/S	28	16.50 ± 0.10	19.04 ± 0.10
L/A	7	16.27 ± 0.33	18.48 ± 0.21
L/L	15	16.66 ± 0.14	19.09 ± 0.15

TABLE VI

DAILY PERCENTAGE GAINS OF NONFOSTERED MICE EXPRESSED ON THE BASIS OF COMPOUND INTEREST

Period	Days	S		A		L	
		Gain, %					
1	0–6	18.6	17.0	17.8	19.1	21.8	21.4
2	6–12	9.6	8.9	11.4	9.6	10.4	10.1
3	12–18	3.5	3.6	4.1	4.2	4.7	4.5
4	18–30	4.2	5.2	3.9	4.7	5.7	6.5
5	30–38	2.6	3.0	3.2	3.2	2.4	2.7
6	30–60	0.2	0.5	0.9	1.0	0.8	1.1
7	60–140	0.2	0.2	0.2	0.2	0.2	0.2

Discussion

The Normal Growth Pattern of Mice

The growth of a mouse may be divided conveniently into four periods: (1) prenatal, (2) birth to D-30, (3) D-31 to D-60, (4) D-61 to senescence.

Growth during the prenatal period is influenced markedly by the site of implantation of the fertilized ovum and by the amount of blood supply that the growing embryo will receive. Marshak (11) has shown that the birth weight varies inversely with the litter size, but the relationship is not constant for all strains. No records were taken for the prenatal period, and the data of this experiment begin the morning after birth. Merton (12) has shown that parturition normally occurs between midnight and four in the morning, so that the D–O weight is really the six- to eight-hour weight, or, as MacArthur has termed it, the "birth-fed weight".

The arbitrary selection of D-30 as the end of the first growth period is not a very satisfactory one since there is no characteristic change in the growth pattern at this time. The point of inflection occurs at about D-36, but the D-30 point has been maintained because it is useful for comparison with MacArthur's extensive data. Furthermore, the D–0 to D-30 period is not a homogeneous one, but is marked by two distinct lag phases. The first lag phase occurs soon after birth and lasts for about two days. Because no observations were made between D-0 and D-2, this early lag phase is not apparent in the graphs. The second lag phase occurs at about D-14 and corresponds with the time of weaning. This is well illustrated in all the graphs and represents the period in which effects produced by fostering are at a maximum.

After the point of inflection has been reached, the growth pattern reaches a plateau and the rate of growth becomes quite uniform for the rest of the life span. There is, however, one small period of retarded growth rate to be found at or around D-44. This short lag phase at the first part of the plateau has been reported by Thompson (15) who finds that it coincides with the time of puberty. Evidence for the existence of this phenomenon was found in both the nonfostered and the fostered groups. Thus, for example, the mean weight of S/S females at D-42, D-44, D-46, D-48, and D-50 is 13.30, 13.38, 13.28, 12.35, and 13.43 gm. These weights represent percentage increases of +0.1%, +0.6%, −0.8%, −0.2%, and +1.4% over the previous weights.

Falconer (3) has demonstrated that both the quantity and the quality of the milk may be genetically controlled. Several of the inbred strains show wide differences in their lactating ability and also in the duration of lactation. Data that will be presented in a subsequent paper will show that wide differences may exist between successive lactations of the same mouse. Further, Enzmann (2) has pointed out that the amount of milk produced increases with the number of suckling young, but not proportionately. The milk supply may be a limiting factor in growth, a superabundance of which is enough to produce individuals twice the normal weight at D-20. Parkes (14) achieved this by suckling mice on rats. MacDowell, Gates, and MacDowell (10), too, have been able to increase greatly the weight of mice by systematically reducing the number in the litter as the lactation period progressed.

MacDowell *et al.* (10) found that the differences of weaning weights between mice raised in small and large litters persist for a long time. On the other hand, Zucker *et al.* (17) find that although rats raised in large litters were smaller than normal at weaning, and rats raised in small litters were larger than normal, by seven weeks both sets of rats were back to normal size.

In Table VI the growth pattern up to D-140 is subdivided into seven periods. During these periods the rate of growth is more or less constant, and may be represented on the logarithmic curves by approximately straight lines. The daily percentage gains during these periods are given on a compound interest scale.

During the first six days the L mice are growing at an average rate of 3.15% faster than the A mice and 3.80% faster than the S mice. At this time there appears to be little difference between the A and S mice. The extra initial capital plus the faster rate of growth during the first four periods give the L strain an advantage that by D-30 results in a body weight double that of A or S strains.

By D-60 most mice have attained sexual maturity and the rate of growth after this time is usually very slow. Note that all three strains are growing at the rate of only 0.2% during the period of D-60 to D-140, thus making the D-60 weight a good criterion of mature weight. There are, however, certain exceptional individuals who do most of their growing after they are 60 days old. Unfortunately there are no data that will show whether this is because they have been held back in their growth and are making a belated attempt to realize their genetic potentialities, or whether this late growth has a separate inherited basis. It is interesting in this connection to note that in the differences that Marshak (11) observed between reciprocal hybrids the differential growth came in this period. It is generally conceded that late growth is mostly due to the deposition of fat (1), but Zucker *et al.* (16) show that true basic growth is taking place and that the surplus adipose tissue found in older animals may be due to heterogonic growth.

Environmental Influences on Body Size

It is a well known fact that growth may be retarded and mature size decreased by starvation diets; the converse is also stated to be true but the evidence for it is less substantial. When the size reached by an inbred animal at maturity is increased over that of its litter mates, it may be explained in two ways: either a higher level of nutrition added an increment of size to that produced by the genetic potential, or else the former level of nutrition was not high enough to allow the full genetic potentialities to manifest themselves. The view that nutrition is the limiting factor is the hypothesis usually accepted. Thus in size inheritance studies it is customary to provide the animals with a high level of nutrition while keeping the rest of the environment as uniform as possible. This procedure allows the size genes an equal opportunity of expressing themselves and of producing as near their maximum characteristic as possible. The one thing that is difficult to standardize is the quantity and quality of the mother's milk. Some attempt at standardizing the quantity can be made by limiting the litter size, but the neurogenic influence of the suckling stimulus on lactation makes this ineffective. Also, the very nature of size inheritance experiments that utilize selected strains of varying body size and milking capacity, make standardization impracticable. Mice of large body size in general have larger litters than small mice. Again, inbred mice are usually poor milkers so that a criterion of standardization is difficult to establish.

It has been pointed out above that mature body weight, as estimated by weight, is our main criterion of genotype in size inheritance studies. But

what are the relative roles of nutrition and genes in producing the adult size of the animal? This experiment has shown that differences in nutrition probably involving changes in both quantity and quality may affect the various stages of growth, and that these effects may be carried over to final size. Furthermore, these effects are not as a rule eliminated by compensatory retardation or acceleration in later growth.

MacDowell *et al.* (10) claim that in mice the increased size acquired in the nursing period is maintained throughout life, whereas Zucker (17) found the contrary to be true for rats. Zucker *et al.* suggest that the difference between MacDowell's results and theirs is not primarily one of species, but may be quite reasonably explained on the basis of the nutritional quality of the stock diets used.

The retarding effects of A and S milk that were carried over to maturity may be explained by assuming that the level of nutrition after D-30 was not high enough to enable the retarded mice to return to the normal base line. Since all mice had access to unlimited food and water, it must also be assumed that the lower level of nutrition resulted from a limited intake perhaps through some influence of fostering on the appetite, capacity, or metabolic efficiency of the fostered mice. It is, however, difficult to apply the same argument to X/L mice, which remained larger, unless it is further assumed that the L type of milk is so superior that it has counteracted all other effects.

Relation of Fostering Results to Size Inheritance

It is quite evident from what has been said above that the effect of genetic and environmental influences on growth rates and body size is poorly understood at the present time. The mode of inheritance is polygenic, which makes it extremely difficult to obtain clear-cut segregant classes. The problem is further complicated by the lack of a measure of the environmental effect on the phenotypic expression and also by the lack of knowledge of the mode and time of the action of the genes. In a simplified picture it would be expected that each gene brings about its phenotypic expression by its control of rate or duration of cell division or expansion, or both, and also by the time of inception of its action. If, for instance, a gene increased growth at the rate of 2% per day for 20 days, then its effect on size will depend on the size of the mouse at the time that that particular gene begins its operation. Even a simplified statement such as this raises a host of other questions such as: Does time of inception of gene action depend upon chronological age, size reached, or an undefined unit of biological time? Is the action of the gene such that it adds a definite increment to the final size, or, does it work so as to produce a cumulative percentage effect, or, are both actions operating simultaneously?

Although the time of inception of gene action cannot at present be studied directly, Green and Fekete (4) give some indirect evidence that in early development the age of the animal is more important than its size. The

results obtained here appear to support this view since the lag phases and point of inflection occur at approximately the same age regardless of the size differences produced by fostering. If this is so, then differences in duration of gene action or of growth processes may be ruled out as major factors in the growth differentials, and an explanation must be sought in growth rates.

Adult size is associated with the amount of substrate available and also with the growth inhibiting processes associated with growth itself. The growth inhibiting processes are chiefly involved in the later stages of growth and may be omitted from this portion of the discussion. In the early stages of growth, histodifferentiation is taking place and is not completed until after D-30. Zucker *et al.* (17) conclude that in the rat this period is not of uniform growth acceleration but the result of five cycles whose effects are superimposed upon one another. Each cycle may start with a slower growth rate accompanying histodifferentiation and the creation of new cells, and be followed by a more rapid growth rate as the cells already formed begin to expand. If histodifferentiation is mainly time-controlled, then the major effects of fostering would have to come during the cell expansion phase.

If this is so, then fostering A and S mice on a superior milk source (L type) may be regarded as leading to a much faster than normal expansion of the cells and the fostered young get ahead of their nonfostered litter mates. When the cells reach full size the growth rate decreases until such time as another wave of cell production and differentiation appears. During this phase the slower growing nonfostered mice tend to catch up and the differences due to fostering are lessened. The whole cycle is then repeated. Thus the differences brought about by fostering appear to depend on two things: (*a*) the number and type of cells produced at each period of differentiation, and (*b*) the length of time between the successive cycles. The more cells produced at the beginning of each cycle, the greater the initial difference between fostered and nonfostered mice. On the other hand, the longer the cycle, the less the final differences between these two groups. If it is assumed that the first cycle after birth has the most cells ready for expansion, and also that it is the shortest, then it would be expected that the greatest differential weight would occur at this time.

The foregoing assumptions would explain the wavelike or cyclic nature of the weight data when the fostered weights are plotted as a percentage of the nonfostered weights (Figs. 4 to 9). With regard to the retarding influences of fostering (L/A and L/S), the reverse effect, but in the opposite phase would be expected provided the cycle was long enough so that the nonfostered mice had to wait for more cells to be differentiated. If this did not occur, then the anticipated differences would be less cyclic in nature, but would become progressively larger as long as histodifferentiation was going on. As soon as the deposition of new tissue in significant amounts ceases, the retarded mice would begin to catch up with their nonfostered litter mates. However, whether they catch up or not would depend on the processes that cause cell expansion to terminate.

In these experiments it appears that early deficits or surpluses tend to be eliminated by D-35. Any difference that is retained at this time usually is carried over to maturity although it may be partly modified by the deposition of fat.

To generalize, the results of these experiments are consistent with the theory that early growth is made up of a number of separate cycles, for the effect of fostering is to cause a sudden increase or decrease in the early part of the cycle, followed by a slower return to normality. All this is based on the assumption that fostering does not stimulate or retard the laying down of cells, but merely affects the rate of growth of the already existing cells. The number of cells that are laid down would depend on the heredity of the mouse, whereas the rate and amount of expansion would depend on the nature of nutrition. Such a theory is consistent with the findings of Gregory and Castle (5), MacArthur and Butler (9), and of others who found that the major inherited size differences were brought about by cell number rather than by cell size.

This experiment produced very little evidence on the time of action of the size genes. The differences due to fostering indicate the obvious fact that the genes responsible for differentiation produce their effects at a certain specified time, and the tendency of these effects to be eliminated by D-35 would indicate that the genes controlling cell expansion cease to operate at this time. As a corollary of this it may be stated that if an increase or decrease in growth is produced and maintained until D-35, then the change would still be present at maturity. There is some evidence that the retarding effects of malnutrition do operate in this way, but so far the evidence is that major increases are not retained.

If the theory of successive growth cycles is not accepted, but the older theory of constant growth is retained, then another problem immediately presents itself. This series of data has demonstrated that fostering produces plus or minus deviations from the normal or nonfostered size. Such deviations do not remain constant but fluctuate widely and are transitory in that they are largely eliminated by D-35. Since both plus and minus differences are eliminated, a compensatory mechanism must be involved. Such a system would fit into an arithmetic concept where each gene adds its own quota to the total. Fostering would merely add this quota more or less rapidly depending upon the superiority or inferiority of the milk source.

This compensatory mechanism would not fit the geometric concept suggested by MacArthur and Butler (9), which implies that the phenotypic expression of polygenes is the result of multiplication rather than the summation of separate gene effects. Here it must be assumed that each growth process works on the capital produced by all former processes, which suggests that the effects of fostering whould be geometrically cumulative up to maturity. This objection is not valid if the theory of successive growth cycles is accepted, since the geometric concept fits the size selection data better than the arithmetic one.

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SOME EFFECTS OF ULTRAVIOLET RADIATION ON SOCKEYE SALMON EGGS AND ALEVINS[1]

By G. Mary Bell[2] and William S. Hoar[3]

Abstract

Ultraviolet radiation of wave lengths from 2800 Å to 3100 Å, generated by a General Electric RS sun lamp, was used to irradiate eggs and alevins of the sockeye salmon (*Oncorhynchus nerka*). Mortality curves are presented for a series of measured dosages. Irradiation of eggs in the later stages of development produced a stimulating effect on the rate of hatching. These premature alevins, which hatched a month before the controls, were abnormal in several respects. The vertebral column was curved downward over the region of the heart, growth was retarded, the yolk was not utilized as rapidly as in the controls and pigmentation was delayed. The mortality was particularly high at the time of hatching and the process was abnormal. Histological examination of irradiated alevins revealed changes in the epidermis and fibroelastic layers of the skin. These were localized to the irradiated regions. Heavy doses produced severe degeneration of the epidermal layer with the formation of granules in the nuclei, the breakdown of the goblet cells, and subsequent desquamation. The changes were less extreme with lower dosages and in these fish a recovery began about 13 days after irradiation. Internal organs and tissues beneath the fibroelastic layers of the skin were not visibly affected by the irradiation.

Introduction

The effects of ultraviolet radiation on man and on lower forms such as bacteria have been extensively studied (5, 11, 14). Little work has been done on the lower vertebrates, particularly aquatic forms. Hinricks (9) produced abnormalities in developing *Fundulus heteroclitus* by irradiation of the eggs during the early stages of development. She pointed out that these abnormalities were not specific for ultraviolet but could be produced by a number of factors such as pressure, temperature, increased salinity, X rays, and so forth. Crowell (4) noted sluggishness, blistering of the skin, and subsequent mortality in brook trout fry and fingerlings treated with ultraviolet radiation. The dosages, used in these experiments, were not standardized. The recent studies of Bonham *et al.* (2) and Welander *et al.* (18) on chinook salmon (*Oncorhynchus tschawytscha*) are most closely related to the work reported in this paper, although, as might be expected, the effects of the X radiation are more extreme. Studies of the effect of ultraviolet on fish will prove interesting from the comparative angle and may be of some practical significance, since it has been suggested that young hatchery fish are sometimes adversely affected by sunlight (7).

Methods

The developing sockeye salmon (*Oncorhynchus nerka*) were irradiated with 275 w. General Electric type RS sun lamps, operating on a 110 v. a.c. The glass of the bulb is of a special type transmitting ultraviolet down to 2800 Å.

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"The lamp is a self-contained unit with a mercury discharge element and a tungsten-filament resistance ballast incorporated within an ultraviolet reflector type bulb" (17).

One of the commonest faults in biological research in which ultraviolet radiations are used has been the lack of standardization of dosages. The total energy should be ascertained by some nonselective method. Pohle's (16) method was used in this study. This is one of the photochemical methods and is based on the fact that iodine is set free from a solution of hydriodic acid to give a blue coloration with the starch test and that a definite amount of energy is required to produce a particular change in color. In the present experiments, the time required for the whole solution to turn blue was measured with a stopwatch. This time period is equivalent to 1 Finsen and is here referred to as "1 dose" or "1 x".

Sockeye eggs were obtained from Weaver Creek, B.C., on Oct. 16, 1948, and reared in the experimental hatchery at the University. We are indebted to J. E. Mason for this material. To perform the irradiation experiments the eggs were removed from the hatchery troughs to aluminum-coated pans (22.5 cm. squared). The pans were fitted with inlet and outlet allowing running water to a depth of 4 cm. This kept the temperature constant within 1° to 3° C. The eggs were placed on the bottom of the pans in single layers and confined to an area of 10 × 10 cm. by means of glass rods. The distance from the glass of the lamp bulb to the bottom of the pans was 26.5 cm. For the control groups a heavy glass plate was placed between the eggs and the lamp and served to filter out all rays below about 3100 Å. Thus, the experimental eggs differed from the controls in being exposed to a band of ultraviolet radiation extending from about 3100 Å to about 2800 Å. This band extends slightly below the ultraviolet of the lower limits of the solar spectrum. After irradiation the eggs were transferred to wire trays and returned to the hatchery troughs.

The above procedure was modified somewhat for the alevin sockeye. These fish were maintained in perforated metal baskets suspended in the hatchery troughs. The water in the baskets was 4 to 5 cm. deep. Lamps could be placed over these baskets and the irradiations thus performed while the fish were in the hatchery troughs. This obviated the necessity for moving the fish with the attendant possibilities of mechanical injuries. The distance of the animals from the lamp was the same, viz., 26.5 cm.

Material used for histological study was fixed in Bouin's picric acid – formol – acetic acid fixative and prepared for sectioning in a routine manner using dioxan. Sagittal serial sections were cut at 5 μ and stained routinely with Harris' haematoxylin and eosin.

Results

Irradiation of Salmon Eggs

Six sets of irradiations were carried out using the developing eggs. The first of these experiments (Nov. 12), involving 200 eggs, was performed two

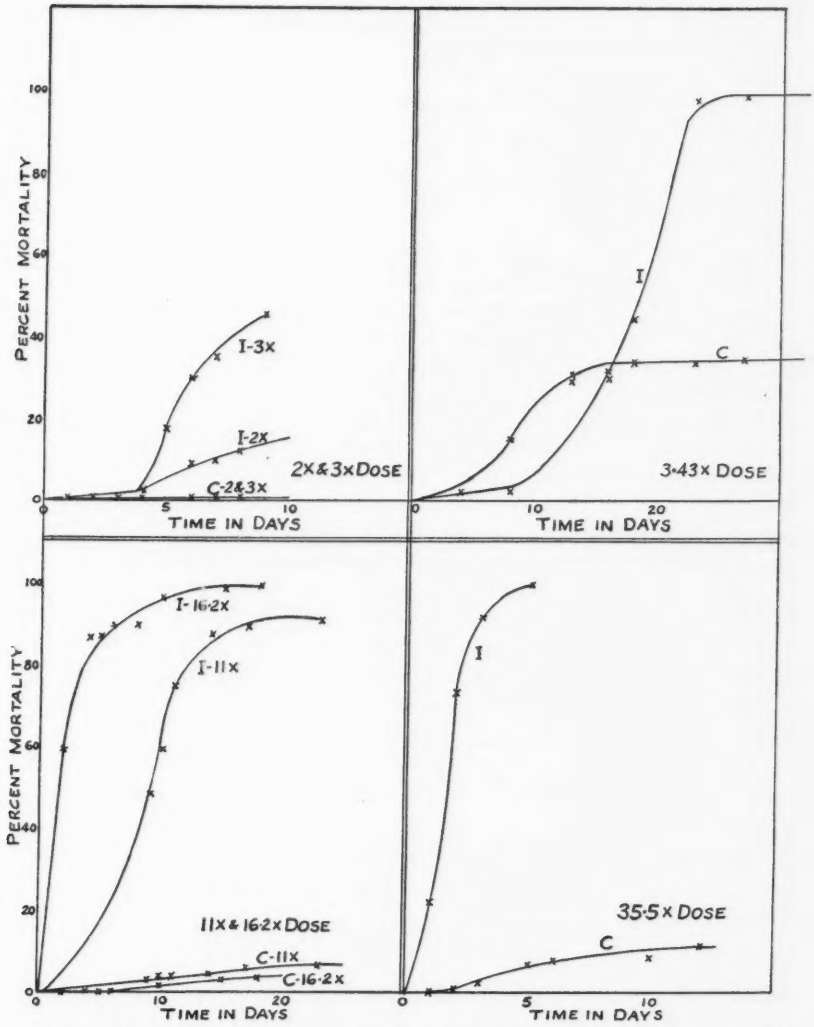


FIG. 1. *Percentage mortalities of irradiated (I) and control eggs (C) following dosages of 2x, 3x, 3.43x, 11x, 16.2x, and 35.5x.*

days before the eggs became "eyed". Other samples were irradiated at intervals of 1 day (80 eggs), 17 days (440 eggs), 20 days (440 eggs), 38 days (400 eggs), and 48 days (400 eggs) thereafter. The dosages used in this series of experiments were 2x, 3x, 35.5x, 16.2x, 11x, and 3.43x—listed in the same order as the irradiation intervals. The numbers of eggs indicated were equally divided between experimentals and controls. The results are summarized graphically in Fig. 1.

The harmful effects of the ultraviolet are evident. Unfortunately the experiments with dosages of 2x and 3x were interrupted before completion but the sigmoid character of the mortality curve is clearly evident at all higher dosages and the steepness of the curve clearly dependent on the intensity of irradiation (Fig. 1). A series of irradiations of chum salmon eggs (*Oncorhynchus keta*), not reported in detail here, showed essentially the same features. Increased mortality in the controls (Fig. 1, dose 3.43x) was due to an attack of fungus on these eggs.

An interesting feature of the irradiations of sockeye eggs on Dec. 20 was the fact that, as well as producing lethal effects, hatching of the eggs was accelerated. The untreated eggs in the hatchery troughs began to hatch on Jan. 18. The irradiated fish began to hatch on Dec. 21, one day after irradiation. At the end of the first week after irradiation half of the eggs had hatched, but with 50% mortality. A number of the survivors of these irradiated fish showed distortions of the body (Fig. 3). These malformed fish were allowed to develop for some time. Figs. 3 and 4 are photographs of irradiated and control sockeye salmon alevins 46 days after irradiation of the eggs. The control fish (Fig. 4) show more development of pigmentation and silvering, increased absorption of yolk, development of fins, and more advanced general development. The irradiated fish (Fig. 3) show distorted body contours with the head depressed over the yolk sac at the level of the heart. The formation of pigment was delayed two weeks in these irradiated fish. Until the time of death, 75 days after irradiation, pigmentation on the dorsal regions of the head was lacking. The irradiated fish were active but could swim only in circles since their bodies were distorted.

Hatching was again stimulated in a similar experiment carried out on Dec. 30, using a dosage of 3.43x. The retardation of development and distortion of the bodies of the irradiated fish was likewise evident. In these experiments, the greatest mortality occurred during the hatching process. The fish emerged head first, body first, or yolk sac first, as opposed to the normal hatching fish, which emerged tail first. Control fish only occasionally showed similar abnormalities in hatching.

Irradiation of Salmon Alevins

Five experimental groups and two control groups of alevins were studied. These fish began hatching on Jan. 18, with a peak on Jan. 27, and were 20 mm. average length when irradiated. The 2x (271 fish), 4x (245 fish), and 8x (220 fish) irradiations were performed on Feb. 4; the 0.5x (341 fish) and 1x (303 fish) irradiations on Feb. 11. Since the water temperature never rose above 3° C. during that time, development was not essentially different and the two sets of results have been considered together. The data are presented graphically in Fig. 2. Control 1 (366 larvae) was carried out with the irradiations of Feb. 4 and control 2 (291 larvae) with those of Feb. 11. The experiments were terminated at the end of 30 days. The fish removed for histological work

were compensated for in calculating the theoretical number of deaths that would have occurred had no fish been removed for this purpose. These values were used in preparing Fig. 2.

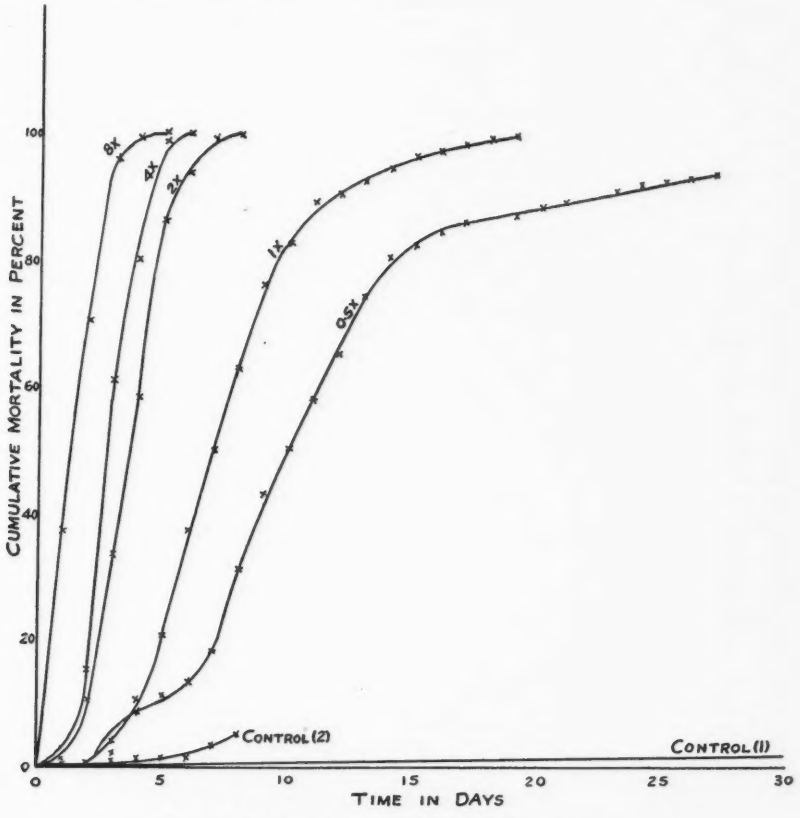


FIG. 2. *Mortality of irradiated alevin sockeye salmon.*

A definite effect on the activity of the fish was noted in all the irradiations carried out using the alevins. During the irradiations the alevins actively crowded into the farthest corners of the holding baskets. In the longer exposures some of the fish became visibly weakened and sank to the bottom of the basket. Some made occasional spasmodic movements, rising swiftly from the bottom to the surface only to fall back and remain quiescent for long periods. These less active fish were observed to be the first to die. Subsequent observations of activity showed a gradation from occasional movements among the 8x group to great activity among the controls. A similar reaction was noted when a bright light was shone on each group. This loss of activity remained in the higher exposures until complete mortality occurred.

In the two lower intensities of irradiation, the fish remained less active than the controls for a period of approximately 15 days. At this time, although most of the fish had died, some recovery was evident in those that remained. At the conclusion of the experiment (30 days), the 17 fish remaining from the 0.5x irradiation showed the same degree of activity as the controls.

In the heavily irradiated groups, a number of effects in addition to the loss of swimming activity were observed. The heart beat became slow and spasmodic. Many seemed unable to move the lower jaw in respiration. White spots appeared on the yolk sac, the vitelline veins were prominent and distended. In many cases, serous fluid accumulated below the lateral line near the attachment of the yolk sac. Mortalities occurred too rapidly and the water temperature was too low to obtain differences in growth rate. However, retardation in skin coloration was noted here as in the case of the irradiated eggs. For example, development of the silvery guanine was delayed about one week in those fish that survived the lower (0.5x and 1x) irradiations.

Histological Findings

Serial sagittal sections of a series of irradiated alevins have been examined. The effect of the ultraviolet on the superficial tissues—described below—is clearly evident. Tissues lying deeper in the fish, however, did not show histological changes after irradiation. The thyroid and pituitary glands were similar to the controls both in development and histological structure. The kidney was apparently unaffected both in the pronephric and mesonephric regions. A qualitative study of the hemopoietic tissue revealed no changes in the nuclei of the hemopoietic cells or in the number of red blood cells in the kidney. Quantitative counts were not made. The thymus gland, gills, nervous system, and digestive tracts were compared in irradiated and control material but no irradiation effects were noted.

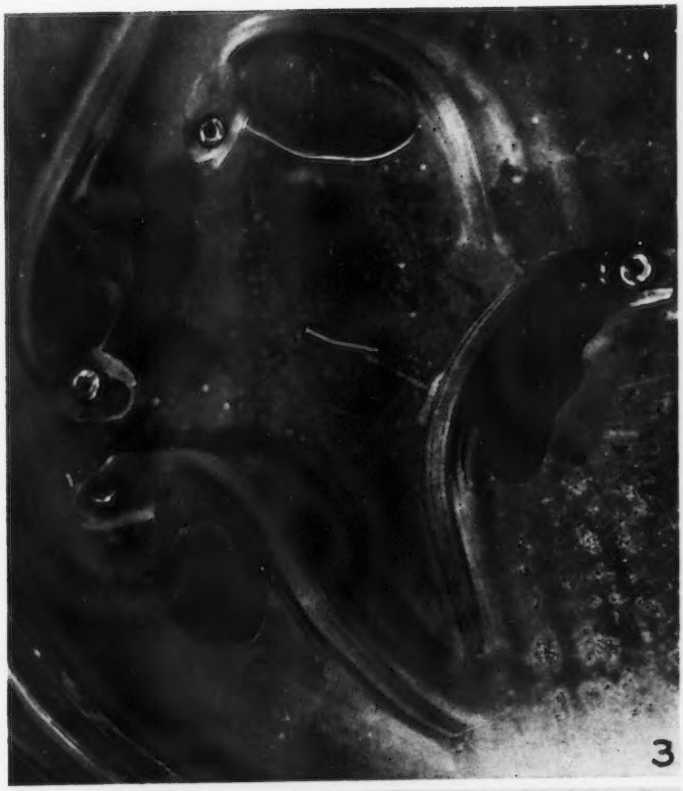
The irradiations produced very definite histological changes in the skin. These changes were not evident during the first two days after irradiation but appeared in both the epidermis and dermis on the third day at all intensities of irradiation. These effects were noted only in the areas directly exposed to the radiant energy. The nuclei of the superficial epidermal cells rounded up into one or two granules which stained variously with acid and basic dyes. The fibroelastic layer of the dermis was swollen and distended. The goblet cells became rounded and the pores opening to the surface were wider than those of the controls (Figs. 5 and 6).

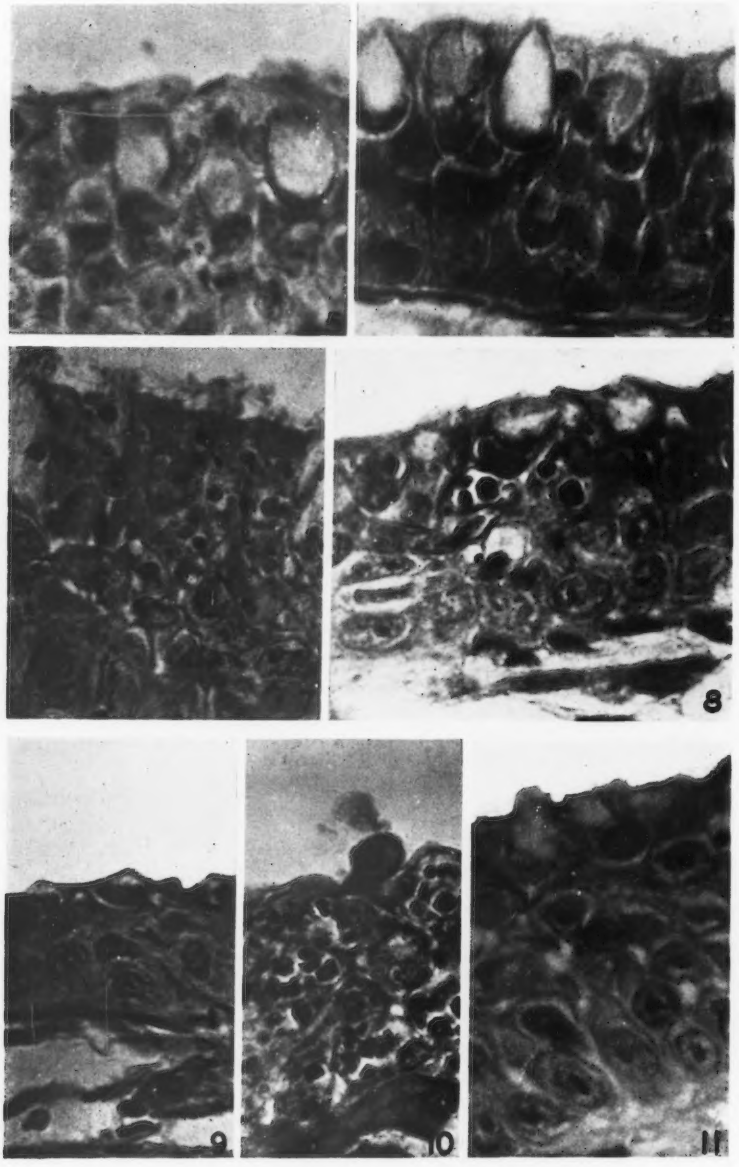
On the fourth day after the irradiation the granular remains of the nuclei were more numerous and could be observed in the basal layers of the epidermis (Fig. 7). The goblet cells were greatly reduced in number and in some areas

FIG. 3. *Sockeye alevins (total length, 23.5 mm.) 46 days after irradiation of 35.5x.*

FIG. 4. *Control sockeye alevins (total length, 24.5 mm.) for experimental fish shown in Fig. 3.*

PLATE I

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completely absent. The skin of the more heavily irradiated fish (4x and 8x) was ragged on the outer surface as though it were desquamating (Fig. 8). Less intense irradiations (0.5x, 1x, 2x) produced granulation and reduction or loss of goblet cells but did not bring about signs of desquamation (Fig. 9).

Evidence of skin damage increased in intensity up to the eighth day after irradiation. Severe degeneration with numerous granulated nuclei is evident after five to eight days at the higher (2x, 4x, 8x) dosages (Fig. 10). The fibroelastic layer, which became swollen on the third or fourth day, was very fragmentary at this time. Death occurred before any recovery was evident in these higher dosages. Lower dosages (0.5x and 1x) showed less severe granulation in the epidermis and less distention of the fibroelastic layer. Here again, the most severe effects were observed on the eighth day. These lasted until the 13th or 15th day when recovery became evident (Fig. 11). The first evidence for recovery was the heightening of the basal layer of the epidermis. Subsequent recovery involved proliferation of these epidermal cells and the appearance of new goblet cells. This recovery is clearly evident by the 18th day.

The observable effect of irradiation on the skin is a purely local one. Sections of skin from the sides of the body that were not exposed to the ultraviolet were similar to the controls (Fig. 6). Goblet cells were normal and the nuclei showed no signs of granulation. The edema was not evident.

Discussion

From the standpoint of a study in environmental physiology, it may be argued that such young fish are never subjected to ultraviolet radiation in nature. This is true but salmon fry are exposed to sunlight and it is well recognized by hatchery workers that care must be exercised in this regard when moving young fish from the hatchery to exposed outdoor ponds. Sufficient experiments were carried out with 5 cm. long coho fry (*Oncorhynchus kisutch*) and 5 to 10 cm. long goldfish (*Carassius auratus*) to show that the irradiations used here were lethal in higher dosages and produced skin injuries (evidenced by growth of fungus and changes in pigmentation) in lesser intensities. Since the intensities used in this study are roughly comparable to those found in nature, some of the effects observed may be significant in the survival of hatchery fry when moved to outdoor ponds or planted

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Transverse sections through the skin of sockeye salmon alevins; haematoxylin and eosin stain; sectioned at 5μ; magnification 440×.

FIG. 5. *Three days after irradiation of 4x.*

FIG. 6. *Control fish, seven days after beginning of experiment.*

FIG. 7. *Four days after irradiation of 2x.*

FIG. 8. *Four days after irradiation of 8x.*

FIG. 9. *Seven days after irradiation of 0.5x.*

FIG. 10. *Seven days after irradiation of 2x.*

FIG. 11. *Thirteen days after irradiation of 0.5x.*

in exposed places. The loss in activity, and the injury of the skin leading to decreased osmotic control or fungus growth are perhaps most important.

From the more theoretical angle three observations arising from this study will bear comment, viz., the effect on the rate of hatching, the mortality, and the histological changes in the skin.

Premature hatching of *Fundulus* eggs exposed to ultraviolet was observed by Hinricks (9). These observations agree with the experiments reported here where large doses at later stages in embryonic development hastened the hatching. The mechanism of the effect is not clear. In some way the radiation must have weakened the chorion so that the embryo moving within ruptured it. Embryonic development was not apparently speeded up since the embryos that hatched were not as fully developed as the controls.

Hayes (8) has shown that there is a preliminary softening of *Salmo salar* egg membranes from between the 25th to 15th day before hatching. It has been shown that this first softening is not due to the hatching enzyme, but the final softening is due to this enzyme. This hatching enzyme appears only a few hours before the egg breaks. The preliminary softening may rupture the capsule but, according to Hayes, will never produce normal hatching.

It is evident that ultraviolet has a lethal effect both on salmon eggs and young fish. The effect is primarily a surface one since ultraviolet is absorbed by thin layers of protoplasm (6, 11). The layer of capillaries on the vitelline membrane is probably of importance since the blood flowing through this region will absorb the radiation. The effects of this absorbed energy may then be transmitted to the organism as a whole (12). Part of the energy is transformed to heat by the activation of the molecules (3). This hyperemia probably produced changes in the blood and other tissues that were harmful to the embryos and hatched fish. Further, there is evidence that injured cells elaborate materials that have definite physiological effects (1). The latent period between exposure and physiological responses may be accounted for by assuming that these physiologically active substances are elaborated by cells at a slow rate. The edema observed in the irradiated alevins was, no doubt, caused by such substances; termed, "histamine-like" substance by Lewis and Zotterman (13) or "leucotaxine" by Menkin (15). In the salmon alevins this edema was particularly evident at the junction of the yolk sac and body wall and appeared with dosages of 2x, 4x, and 8x. A similar reaction was observed by Welander *et al.* (18) in the chinook salmon treated with X rays. Thus, the mortalities may be attributed to heat hyperemia caused by absorption of the rays and the development of toxins by the damaged cells. These toxins are probably responsible for permeability changes in capillaries and surface membranes that lead to edema.

The histological changes occurring in irradiated human skin have been carefully described (10). Changes in the salmon skin are similar in that injury and death of the surface cells is followed by a regeneration from the basal layers of the epidermis as recovery takes place. This damage may be

more serious for an animal living in an aquatic habitat. The destruction of the goblet cells and resulting loss of mucus may lead to an abnormal osmotic exchange through the surface layers. The accumulation of fluid in the fibroelastic layers may be interpreted in this way or may be due to the toxic substances referred to above, which in turn affect capillary permeability.

It would seem that the superficial epidermal cells were completely destroyed. Granulation appeared first in the nucleus and secondarily in the cytoplasm but the entire cell was affected. The wave-lengths used were in the region that Giese (6) found to be most highly absorbed by protoplasm. If the nuclei of the germinal cells of the epidermis were not injured or destroyed recovery took place. It would be interesting to know whether the fish skin could develop increased resistance to radiation by a series of exposures and, if so, what the nature of this protection might be. Stimulation of melanin production was observed in irradiated goldfish. This is in the nature of an acclimatization reaction but it is probable that changes in the character of the cell protoplasm and in the thickness of the epidermis might also occur as in man (1, 11, 12).

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